The Journal of
CLINICAL
ENDOCRINOLOGY

VOLUME 11 JANUARY, 1951 NUMBER 1
Copyright 1951 by the Association for the Study of Internal Secretions

TREATMENT OF CONGENITAL ADRENAL HYPERPLASIA WITH CORTISONE*

LAWSON WILKINS, M.D., ROGER A. LEWIS, M.D., ROBERT KLEIN, M.D., LYTT I. GARDNER, M.D., JOHN F. CRIGLER, JR., M.D., EUGENIA ROSEMBERG, M.D.† AND CLAUDE JEAN MIGEON, M.D.‡

From the Department of Pediatrics, Johns Hopkins University School of Medicine and the Harriet Lane Home of The Johns Hopkins Hospital, Baltimore, Md.

CONGENITAL adrenal hyperplasia causes pseudohermaphrodism in females and macrogenitosomia precox in males. The differentiation of these conditions from other types of intersexuality and from various disorders causing male sexual precocity is well known (1). This type of hyperadrenocorticism in contrast to the Cushing type is characterized by accelerated growth, muscular development and epiphysial ossification, early growth of sexual hair and progressive virilization, all of which are due to excessive secretion of adrenal androgen. In most cases there are no evidences of disturbance of electrolyte or carbohydrate regulation; in

Received for publication November 21, 1950.

* This work was made possible by grants-in-aid from the American Cancer Society and the United States Public Health Service.

† Argentine recipient of the Mead Johnson Fellowship of the Society for Pediatric Research.

‡ Dr. Migeon's cooperation in this investigation was made possible by a maintenance grant from the United States Government under the Smith-Mundt Act, and by a grant from the American Field Service Fellowships for French Universities, Inc. Both grants were awarded to Dr. Migeon upon the recommendation of the Institute of International Education.

others there is a disorder of electrolyte regulation causing symptoms similar to those of Addison's disease and often leading to death.

Unsuccessful attempts (unpublished) have been made by us to suppress the secretion of androgen in patients with congenital adrenal hyperplasia by the administration of steroids, such as 17-ethyl testosterone, 17-vinyl testosterone, 17-methyl androstenediol and 17-methyl androstanediol, which have a chemical structure similar to that of androgens but possess relatively little androgenic activity. Treatment of patients with cortisone (11-dehydro-17-hydroxycorticosterone acetate) was begun in January 1950 and preliminary reports of the first cases have been published (2, 3). This paper presents in more detail the results we have observed following the administration of cortisone to 6 females and 2 males with congenital adrenal hyperplasia and includes the findings of other observers in 2 additional cases.

Case 1. P. R. (A 58433) was a female pseudohermaphrodite, 15 years of age. At the age of $6\frac{1}{4}$ years, her left adrenal and a portion of the right were removed by Dr. Hugh H. Young without influencing the progressive virilization and hirsutism. During the seven years before this study, repeated determinations of the urinary 17-ketosteroids showed daily excretions ranging from 20 to 68 mg. per day, with an average of 42 mg. per day.

As shown in Figure 1, the excretion of 17-ketosteroids during the control period of ten days varied between 32 and 68 mg. per day, with an average of 48 mg. per day. She was then treated for fifteen days with cortisone given intramuscularly at 6-hour intervals in doses amounting to 100 mg. daily. The 17-ketosteroids decreased sharply on the second day of treatment and after the fourth day remained at a level between 4.0 and 8.4 mg. per day, with an average of 6.4 mg. per day, throughout the remaining period of treatment. The output of 17-ketosteroids did not increase until ten days after treatment was discontinued and then rose to levels between 28 and 41 mg. per day and later returned to the pretreatment level.

The urinary estroids, determined by the fluorometric method of Jailer (4), followed the same pattern as the 17-ketosteroids. They decreased from a pretreatment level of 33 to 44 micrograms per day to levels of 8 to 10 micrograms per day during treatment with cortisone and rose to 23 micrograms per day by the thirteenth day after discontinuing treatment.

The excretion of corticosteroids (designated in the figures as "11-oxysteroids")[1] varied between 1.39 and 1.74 mg. per day, with an average of 1.61 mg. per day, before treatment. On the first day of treatment the output decreased to 0.58 mg. per day and during the first ten days of treatment remained between 0.37 and 0.51 mg., with an average of 0.44 mg. However, on the twelfth and fourteenth days of treatment the excretion increased to 1.04 and 1.07 mg. respectively. We believe that this rise was probably due to the excretion of a portion of the cortisone administered. On discontinuing treatment, the output gradually decreased to 0.58 mg. by the seventh day, rising to levels of 0.67 to 0.83 mg. at the end of the study.

---

[1] The corticosteroids of the urine were measured by the phosphomolybdic acid color reaction (5) performed on extracts obtained by chloroform extraction and subjected to benzene-water partition (6) but not Girard's separation.

Case 2. W. J. (A 68429) was a 2½-year-old boy with macrogenitosomia precox. The diagnosis of congenital adrenal hyperplasia was confirmed at the age of 22 months by exploration of both adrenals. The left adrenal, weighing 16 grams, and the left testis, containing aberrant adrenal tissue, were removed at this time. At no time had this boy shown any evidence of an electrolyte disturbance. However, on one occasion in the course of an acute infection he showed a tendency to hypoglycemia. Another unusual

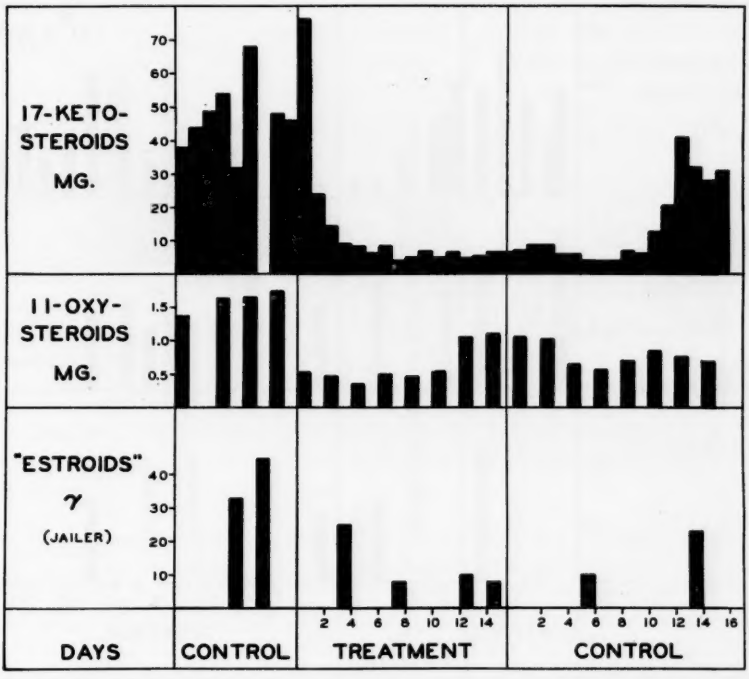

FIG. 1. Effects on urinary steroids of treatment with 100 mg. of cortisone daily in a 15-year-old female pseudohermaphrodite with congenital adrenal hyperplasia.

symptom in his case was marked hypertension which had varied between 150/100 and 160/110 and on several occasions had led to cardiac decompensation.

Prior to treatment the average urinary output of 17-ketosteroids was 9.6 mg. per day; the corticosteroids 1.25 mg. per day; and the estroids 24 micrograms per day. With daily doses of 50 mg. of cortisone (25 mg. every twelve hours) the 17-ketosteroids decreased by the eighth to tenth day to 1.4 mg. per day, the corticosteroids to 0.94 mg. and the estroids to 15 micrograms (Fig. 2). On the tenth and eleventh day the patient became excited to the point of mania and the blood pressure rose to 210/140. Accordingly treatment was discontinued. Following the withdrawal of treatment there was a gradual increase of the 17-ketosteroid excretion, but it is difficult to interpret the findings during this period because of intervening respiratory infections.

Case 3. W. D. (A 30453) was a female pseudohermaphrodite of 8½ years. During the control period the urinary 17-ketosteroids fluctuated between 30 and 70 mg. per day, with an average of 48 mg. per day. The corticosteroids averaged 1.57 mg., the estroids 62 micrograms per day. The administration of 25 mg. of cortisone daily (12.5 mg. given at 12-hour intervals) was followed by a decrease of the 17-ketosteroids to 9 mg. by the sixth day, and a corresponding decrease in the corticosteroids and estroids. On

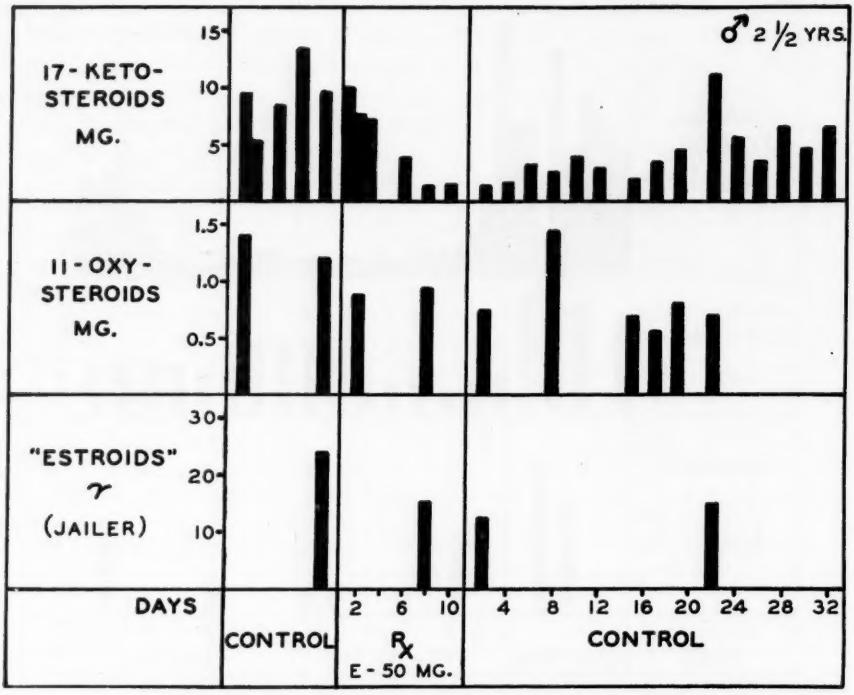

FIG. 2. Effects on urinary steroids of treatment with 50 mg. of cortisone daily in a male with congenital adrenal hyperplasia.

the tenth day, however, a slight cold and fever developed. The 17-ketosteroid excretion rose abruptly to a peak of 52 mg. on the following day, and then decreased to a level of 6 to 8 mg. during the next four to five days. The excretion of corticosteroids and estroids showed similar rises during this slight illness. After this the output of 17-ketosteroids remained at an average level of 5.3 mg. per day, except for occasional slight rises to 13 or 14 mg. There was no tendency for the suppression to become less effective during the forty-five days of treatment.

We believe that in this case the rise of 17-ketosteroids, corticosteroids and estroids under the stress of a slight illness is of considerable interest. It is probable that the small dose of 25 mg. of cortisone was sufficient to suppress the abnormal adrenal activity in this patient under normal conditions of life but that the stress of the acute illness caused

sufficient secretion of ACTH to cause again an outpouring of steroids from her hyperplastic adrenals. We attempted to test the patient's responsiveness to 80 mg. of ACTH (given in divided doses every four hours) on the thirty-ninth day, but at that time there was relatively little change in the secretion of urinary steroids with this dose (Fig. 3).

Case 4. M. N. (A 76987) was a female pseudohermaphrodite of 9½ years whom we were able to study through the courtesy of Dr. Lawrence Kyle. Before treatment the output of 17-ketosteroids varied between 24 and 33 mg. per day, with an average of 28 mg. per day. The average daily output of corticosteroids was 0.87 mg. The urinary

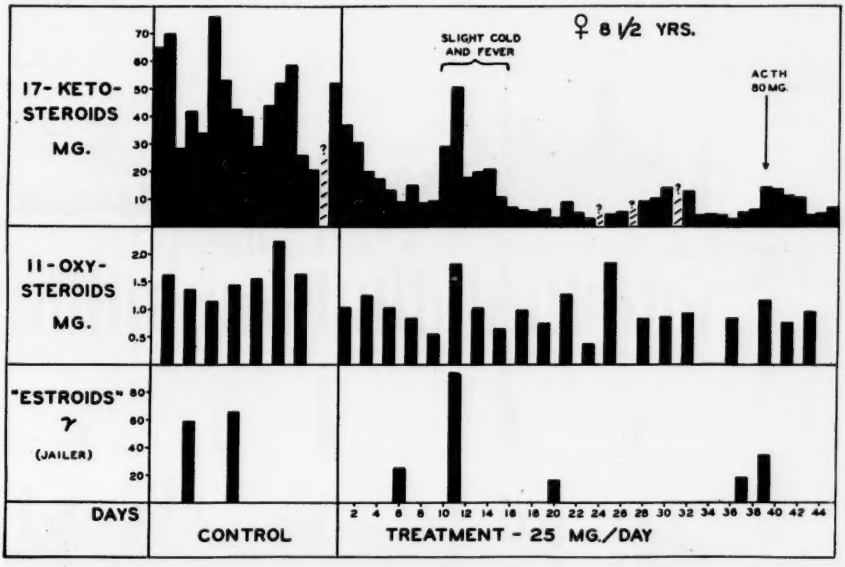

FIG. 3. Effects on urinary steroids of treatment with 25 mg. of cortisone daily in a female pseudohermaphrodite with congenital adrenal hyperplasia.

estroids were 32 micrograms on one determination. Prior to treatment with cortisone the effects of Δ^5 pregnenolone (Δ^5-pregnene-3β-ol-20-one) given in doses of 100 to 300 mg. per day were studied. This period of treatment was interrupted by a slight digestive upset lasting three or four days, during which there was an increase in the excretion of 17-ketosteroids to 54 to 56 mg. per day. The pregnenolone caused no diminution in the output of any of the urinary steroids. Following the course of pregnenolone, the administration of cortisone in doses of 25 mg. given once a day caused a decrease in the urinary 17-ketosteroids over a period of several days to levels between 8 and 14 mg. per day. Continuation of this dose until the thirty-third day resulted in no further decrease. The amount of cortisone given was then increased to 50 mg. daily (25 mg. twice a day) following which the output of 17-ketosteroids decreased further to levels between 4.5 and 7.5 mg. per day.

The effects of 85 to 100 mg. of ACTH (given in four doses at 6-hour intervals) were tested before treatment with cortisone and at varying intervals while cortisone was

being given. As shown by Figure 4, ACTH caused slight increases in the output of urinary steroids while a daily dose of 25 mg. of cortisone was being given, but very little response when the dose of cortisone was increased to 50 mg. daily.

Case 5. M. B. (A 47344) was a female pseudohermaphrodite of 8½ years. During the control period the output of 17-ketosteroids varied between 37 and 43 mg. per day, with an average of 38 mg. per day; the corticosteroids were 1.47 and 1.05 mg.; and the estroids 105 micrograms per day. Because in the previous case the administration of 25

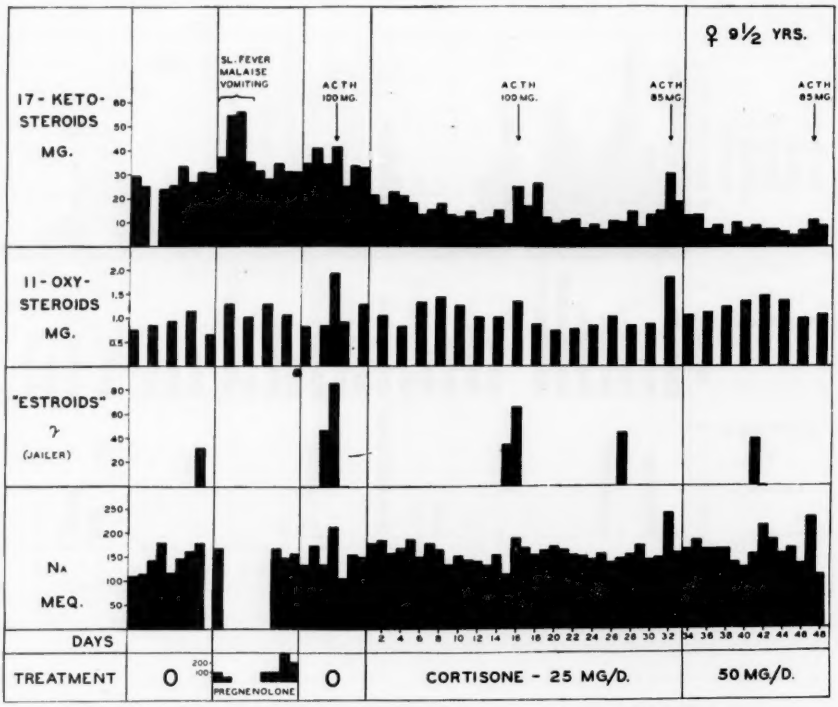

FIG. 4. Urinary steroids and sodium excretion in a female pseudohermaphrodite treated with Δ^5 pregnenolone and with cortisone. Responsiveness to ACTH was tested before and during cortisone therapy.

mg. of cortisone daily had failed to reduce the urinary 17-ketosteroids below a level of 10 to 14 mg. per day, it was decided to treat this patient first with 100 mg. of cortisone given once a day to cause a rapid and more marked decrease in the output of 17-ketosteroids and then to attempt to maintain the suppression with doses of 25 mg. daily. As shown by Figure 5, this plan was successful. The urinary 17-ketosteroids fell to 28 mg. on the first day of treatment and by the sixth day had decreased to 5 mg. On the seventh day the dose of cortisone was reduced to 25 mg. daily given in a single dose. This treatment has now been continued for 150 days, being administered at home since the twenty-sixth day. During this time the 17-ketosteroid output has varied between

2.5 mg. and 8.5 mg. except that it increased to 8 to 12 mg. at one period when the mother reduced slightly the dose of cortisone. There seemed to be a slight tendency for the urinary excretion of sodium to decrease with 100 mg. of cortisone but the output rose again when the dose was decreased to 25 mg. About one month after treatment was begun, the breasts began to develop. They have grown gradually larger and now measure 8 cm. in diameter. The vaginal smear shows estrogenic changes. Acne has decreased.

Case 6. S. B. (A 73200) was a male infant of 23 months with macrogenitosomia precox combined with Addisonian-like symptoms. During the first four months of life, there had been vomiting, failure to gain weight and persistent dehydration. At that age, studies made by Dr. John E. Gonce, Jr., at the University of Wisconsin, revealed a serum sodium of 115 mEq./L., a potassium of 7.6 mEq./L. and urinary 17-keto-

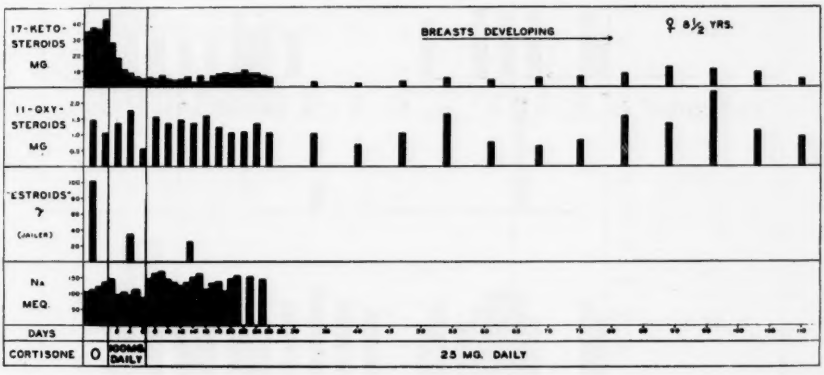

FIG. 5. Urinary steroids and sodium excretion in a female pseudohermaphrodite treated initially with 100 mg. of cortisone daily for six days and then with 25 mg. of cortisone daily. Treatment has been continued for five months. Development of breasts and estrogenic changes in the vaginal smear began after seven weeks and increased progressively.

steroids of 4.2 mg. per day. These findings led to a diagnosis of congenital adrenal hyperplasia with a disturbance of salt regulation. From this time on, the child was maintained on a high intake of NaCl and was given desoxycorticosterone in doses of 1.75 mg. every second or third day. In October 1949 he was studied at the Harriet Lane Home and two pellets of desoxycorticosterone, weighing 125 mg. each, were implanted and injections of the hormone were discontinued. With a daily intake of approximately 5 Gm. of NaCl, he had remained well until the time of this study in May 1950 when he was 23 months old. At this time he presented a typical picture of macrogenitosomia precox. The serum sodium was 146 mEq./L. and potassium 5.0 mEq./L. During the control period the urinary 17-ketosteroids varied between 16 and 36 mg. per day, with an average of 29 mg. per day. The corticosteroids averaged 0.87 mg. per day. The administration of 25 mg. of cortisone given once a day caused a rather slow fall in the output of 17-ketosteroids, which decreased to a level of 5.8 mg. by the eleventh day. Urinary estroids dropped correspondingly but there was little effect on the corticosteroids. During treatment no effect on the urinary sodium or potassium excretion or the serum sodium

and potassium was observed, but between the second and fourth day after withdrawal of cortisone there was a marked sodium diuresis suggesting that cortisone may have been acting as a salt-retaining hormone (Fig. 6).

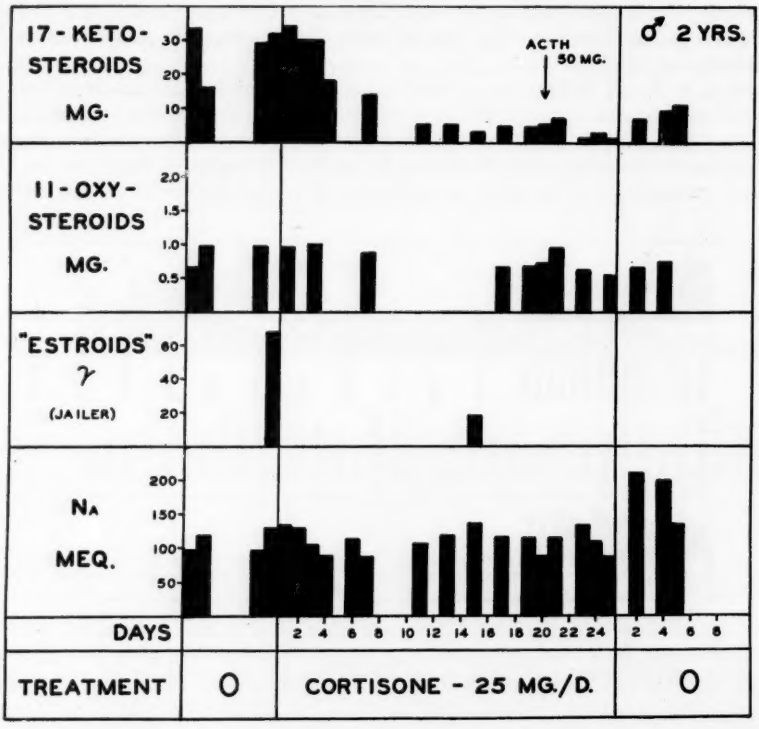

FIG. 6. Effects of cortisone on urinary steroids and sodium excretion in a male with congenital adrenal hyperplasia who had previously shown Addisonian-like symptoms. Six months prior to cortisone therapy pellets of DCA had been implanted and were still palpable, but no additional DCA was administered during the study.

Case 7. J. S. (92456) was an 18½-year-old girl with female pseudohermaphrodism who had been under observation at The Johns Hopkins Hospital since the age of 3 years. A 16-year-old sister has the same condition. At 5 years the diagnosis was confirmed by adrenal exploration and a biopsy specimen was taken from an ovary. At 6 years the clitoris was amputated and the urogenital sinus was corrected to form a separate vagina and urethra. There was progressive virilization throughout childhood. Between 1941 and 1943, when the patient was 9 to 11 years of age, she had been treated with stilbestrol. The breasts developed slightly and scanty menstrual spotting occurred on two occasions. After discontinuing stilbestrol the breasts disappeared and no further menstrual bleeding occurred. At various times during childhood blood pressure readings from 125/65 to 150/90 were recorded. Six months prior to treatment hypertension, varying from 170/115 to 210/150, and slight cardiac enlargement were found by Dr.

John E. Howard. Dr. Frank Walsh observed slight sclerosis of the retinal arteries. The results were negative for the benzodioxane test and normal for renal function tests. The use of a salt-poor diet resulted in no significant lowering of blood pressure.

The studies with cortisone were begun in June 1950 in cooperation with Dr. John E. Howard. When the patient was examined prior to treatment, there was a diffuse brownish pigmentation of the skin, with accentuation in the perianal region, in the lines of the palms, and over the knuckles, resembling that of Addison's disease. There was also dark brown pigmentation of the upper gums. The nipples were dark and were

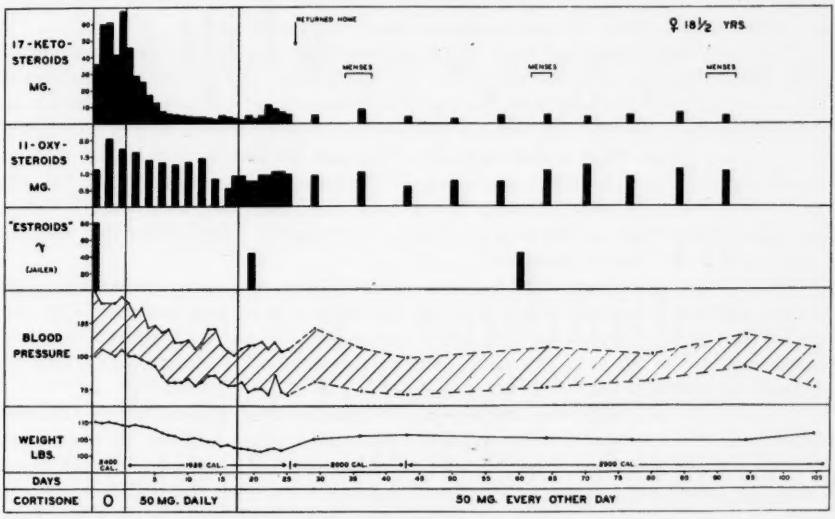

FIG. 7. Effects of cortisone therapy in a female pseudohermaphrodite of 18½ years who had hypertension and Addisonian-like pigmentation. Treatment has now been continued for five months. Breasts have developed, and four menstrual periods have occurred. Pigmentation has decreased.

surrounded by scattered coarse hairs. There was a moderate amount of padding of fat in the breast region, but no definite mammary tissue was felt. During the control period the blood pressure remained constantly 140 to 150, systolic, and 100 to 105, diastolic. Serum nonprotein nitrogen, fasting sugar, sodium, potassium, chloride and CO_2 combining power were normal. The eosinophil count varied from 121 to 231 per cu. mm. The daily output of 17-ketosteroids ranged between 36 and 68 mg., with an average of 53 mg.; the corticosteroids 1.12 to 2.07 mg., with an average of 1.65 mg.; and the urinary estroids 93 micrograms.

During the study in the hospital the patient was ambulatory. Her activities, diet and salt intake were kept approximately constant. Treatment was begun with 50 mg. of cortisone injected daily. The urinary 17-ketosteroids decreased to 7.6 mg. by the sixth day of treatment and remained below this level until the seventeenth day, the average being 4.3 mg. per day. On the eighteenth day treatment was changed to 50 mg. of cortisone injected *every other day*. As shown by Figure 7, there was a slight rise in the excretion of

17-ketosteroids during the first week, the maximum being 11 mg. per day. This may have been due to the fact that one dose was omitted by mistake the day preceding the maximum rise. Treatment with cortisone on alternate days has now been continued for 140 days. Since the twenty-fourth day the 17-ketosteroids have not exceeded 8 mg., the average of 10 determinations being 5 mg. The effect of treatment on the excretion of corticosteroids was less striking. There seemed to be some decrease, but irregular fluctuations occurred.

The blood pressure remained constantly between 140/100 and 150/105 during the control period in the hospital. During the first fifteen days of treatment it fell progressively to 104/78 and has remained at approximately this level, in spite of the fact that she returned home to a normal regimen on the twenty-sixth day.

Previously the patient had had scanty menstrual spotting on only two occasions in 1941–1943 while receiving stilbestrol. On the thirty-third day of cortisone treatment she menstruated and subsequently has had three more menstrual periods beginning on the sixty-second, ninety-third, and one hundred and twenty-second days of treatment. The flow has lasted three to four days each time and has been normal in amount and unaccompanied by pain. Daily recordings of the basal temperatures during the last cycle showed no significant changes. The breasts have developed considerably during the period of observation. The mammary tissue now measures 7 cm. in diameter, whereas none could be felt before treatment.

All observers agree that there has been striking decrease of skin pigmentation. The patient and her 16-year-old sister, who also has congenital adrenal hyperplasia accompanied by marked pigmentation and hypertension, have led the same life and have had equal exposure to the sun. The sister, who has received no treatment, has shown no decrease of pigmentation.

Case 8. L. U. (A 79277) was a female pseudohermaphrodite with evidence of marked adrenal insufficiency. At birth she weighed 3,160 grams. When feedings were begun on the second day, vomiting occurred after each nursing and continued intermittently thereafter in spite of changes of formula. The stools were small but not loose. On the eighteenth day the weight had decreased to 2,440 grams. The patient became comatose, and the skin was cold and clammy with dusky mottling. She was admitted to a hospital in Morgantown, West Virginia, where she was put in an oxygen tent and given Ringer's solution parenterally. During the next three weeks she was able to retain thick feedings of cream of wheat and Similac but required subcutaneous infusions of salt solution daily. On admission to the Harriet Lane Home at 7 weeks of age the infant weighed 2,600 grams and presented a picture of marked emaciation and dehydration. Chemical studies on the serum at the time of admission (not shown in Figure 8) revealed: sodium 130 mEq./L., potassium 9.0 mEq./L., chloride 112 mEq./L., CO_2 18.3 mEq./L., and nonprotein nitrogen 50 mg. per 100 cc., with a hematocrit reading of 42 per cent. The fasting blood sugar was 96 mg. per cent. During the first twenty-four hours, a total of 965 cc. (370 cc./kilo.) of fluid containing 90 mEq. of sodium was given. Of this amount, 405 cc. were given intravenously and 555 cc. by mouth. Following this the hematocrit decreased to 31 per cent and the nonprotein nitrogen to 18 mg. per cent. The serum sodium was 128 mEq./L., potassium 6.4 mEq./L., chloride 103 mEq./L., and CO_2 18.3 mEq./L.

For four weeks prior to treatment with cortisone, the infant was maintained in a state of fairly good hydration by the administration of large amounts of NaCl (averaging 7 Gm. of NaCl or 120 mEq. of Na per day) first supplied parenterally and orally but later taken entirely in her formula. During this time the weight increased from 2,600

to 3,760 grams but the abnormal pattern of serum electrolytes was not significantly altered (Fig. 8). Eleven determinations of the 17-ketosteroids in the urine revealed values varying from 2.4 to 4.4 mg. per day and averaging 3.5 mg. per day. The corticosteroids were 0.68 mg. per day on two occasions.

Following the prolonged control period, the intake of sodium was maintained at the same level of approximately 120 mEq. per day and the daily administration of 25 mg. of cortisone was begun. By the fourth day the output of 17-ketosteroids decreased to 0.5 mg. per day and from then on remained at levels of 0 to 0.7 mg. per day, the average

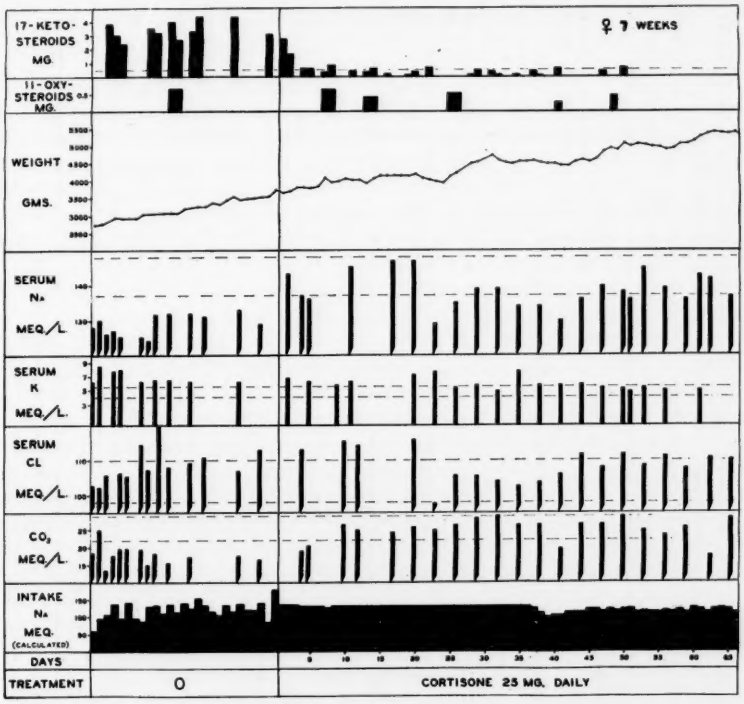

FIG. 8. Effects of high sodium chloride intake and cortisone therapy on urinary steroids and serum electrolytes in a female pseudohermaphrodite of 7 weeks who showed Addisonian-like symptoms with severe dehydration.

of 21 determinations being 0.3 mg. There was no striking change in the excretion of corticosteroids. As shown in Figure 8, following the administration of cortisone there was a distinct tendency for the serum sodium and CO_2 to be maintained at higher levels while the serum chloride remained essentially unchanged. The serum potassium remained at levels of 4.9 to 6.7 mEq./L. with a definite fall to normal levels after prolonged therapy. However, unexplained fluctuations in the serum electrolytes occurred. During the sixty days since cortisone was begun the infant's weight has increased from 3,760 to 5,400 grams. She has become somewhat obese. The face has become definitely

TABLE 1. SUMMARY OF EFFECTS OF CORTISONE THERAPY ON URINARY 17-KETOSTEROIDS, CORTICOSTEROIDS AND ESTROIDS IN CASES OF CONGENITAL ADRENAL HYPERPLASIA

Case	Sex	Age (yrs.)	Complications	Treatment with cortisone			Urinary 17-ketosteroids		Urinary corticosteroids		Urinary estroids	
				Dose mg./day	Time for full effect (days)	Duration of treatment (days)	Before treatment mg./day	During treatment mg./day	Before treatment mg./day	During treatment mg./day	Before treatment, micrograms/day	During treatment, micrograms/day
1. P.R.	F	15		100	6	15	42.0	6.4	1.61	0.44→1.05	38	8
2. W.J.	M	2½	Hypertension	50	4	10	9.6	1.4	1.25	0.94	24	15
3. W.D.	F	8½		25	10	45	48.0	5.3	1.57	1.00	62	17
4. M.N.	F	9½		25 50	15 4	33 25	28.0	11.8 7.1	0.87	1.03 1.26	32(?)	47 39
5. M.B.	F	8½		100 25	5	6 150	38.0	5.7 6.3	1.26	1.25 1.23	105	35 29
6. S.B.	M	2	Salt loss	25	11	24	29.0	4.0	0.87	0.69	67	16
7. J.S.	F	18½	Hypertension Pigmentation	50q.d. 50q.2d.	6	17 140	53.0	4.3 5.0	1.65	1.25	93	40
8. L.U.	M	11 wks.	Salt loss	25	4	60	3.5	0.3	0.68	0.42	23	
A*	M	9 mos.	Salt loss	15 50 25→10	5	9 11 86	8.5	6.3 2.5 1.2–1.6	0.74			
B†	F	5 wks.	Salt loss	15+2.5 mg.DCA		6	6–12	1.8–2.5				

* Case A is a patient treated by Dr. Harold K. Faber and his associates at Stanford University Hospital.
† Case B is a patient treated by Drs. A. Prader and Guido Fanconi at Kinderspital, Zurich.

round with increase in fat pads so that it resembles somewhat the facies of Cushing's syndrome, although it is not ruddy. The fasting blood sugars have remained within normal limits. Her appearance suggests that probably the dose of 25 mg. of cortisone is excessive for her needs.

Additional studies are under way to measure more definitely the effects of cortisone on the electrolyte regulation and to determine whether smaller doses of cortisone given either alone or with desoxycorticosterone are best in her case.

Additional cases studied by others

We have been permitted to include in our series 2 patients treated with cortisone by other workers.

Case A. Dr. Harold K. Faber and his associates at Stanford University Hospital studied a male infant with congenital adrenal hyperplasia who had severe and nearly fatal Addisonian crises shortly after birth. These were controlled by desoxycorticosterone acetate given by injection and later by pellet implants. At 7 months the urinary 17-ketosteroids were 8.5 mg. per day and the corticosteroids 0.74 mg. per day. Cortisone given in doses of 15 mg. daily for nine days did not reduce the 17-ketosteroids below 6.3 mg. per day but when the dose was increased to 50 mg. the output decreased to 1.2 mg. daily. Following this, the dose of cortisone was reduced gradually to 10 mg. daily without causing any increase in the output of 17-ketosteroids. No additional treatment with desoxycorticosterone has been required, but the pellets were still palpable.

Case B. Dr. A. Prader (19), on the service of Dr. Guido Fanconi in Zurich, treated a female pseudohermaphrodite who was admitted to the hospital at 9 days of age because of persistent vomiting and weight loss and dehydration since birth. The total serum base was normal, the chloride low and the potassium high. The dehydration was not corrected by sodium chloride infusions, but with desoxycorticosterone acetate and increased NaCl intake, the vomiting stopped, the weight increased and the serum electrolytes returned to normal. At 5 weeks, while she was receiving 2.5 mg. of desoxycorticosterone daily and 3 grams of NaCl added to her diet, treatment with 15 mg. of cortisone daily was begun. Within four days the urinary 17-ketosteroids decreased from 6–12 mg. per day to 1.8–2.5 mg. per day. Treatment could be continued for only six days as the infant was removed from the hospital. During this time the urinary chloride and sodium did not change, but nitrogen and potassium were increased.

DISCUSSION

The findings are summarized in Figures 1–8 and Table 1.

Effects of cortisone on excretion of 17-ketosteroids

In every case cortisone caused marked decrease in the output of urinary 17-ketosteroids. In the older children (Cases 1, 3, 4, 5 and 7), who had pretreatment values of urinary 17-ketosteroids averaging from 28 to 53 mg. per day, treatment with 25 mg. of cortisone daily reduced the output to as low levels (4 to 8 mg. per day) as did daily doses of 50 mg. or 100 mg. with the exception of 1 patient (Case 4). In this case the daily excretion of 17-ketosteroids fell to only 8 to 14 mg. after thirty-three days of treatment with 25 mg. of cortisone, but dropped to lower levels when the dose

was increased to 50 mg. daily. In this group of patients the rapidity of the reduction of 17-ketosteroids seemed to depend somewhat on the dosage of cortisone. With 50 mg. a day or more, the maximum reduction was attained in four to six days, whereas in Patient 3, who received only 25 mg. daily, ten days elapsed before the maximal effect was attained.

Of the 5 younger infants, 4 (Cases 2, 8, A and B), had daily outputs of 17-ketosteroids ranging from 3.5 to 9.6 before treatment, and 1 infant of 23 months (Case 6) excreted an average of 29 mg. per day. Two of these patients (Cases 2 and 8) were treated respectively with 50 mg. and 25 mg. of cortisone daily and had a prompt drop of the 17-ketosteroid output to levels of 1.4 and 0.3 mg. respectively, while a third (Case 6) whose 17-ketosteroid output was much higher showed a more delayed fall on 25 mg. daily. Patient A, who was treated initially with 15 mg. of cortisone, showed no decrease of 17-ketosteroids in the course of nine days. An increase of dose to 50 mg. resulted in a prompt fall from 7 mg. to 1.6 mg. per day. Subsequently the dose of cortisone was reduced to 10 mg. per day without causing any increase in the 17-ketosteroid excretion. In the fifth infant (Patient B) who was receiving 2.5 mg. of desoxycorticosterone daily, additional treatment with 15 mg. of cortisone caused a drop of urinary 17-ketosteroids from 6–12 mg. per day to 1.8–2.5 mg., but the patient was followed for only six days.

In 6 patients (Cases 1, 2, 3, 4, 6 and B) only preliminary studies were made with periods of treatment ranging from six to forty-eight days. In Cases 5, 7, 8 and A, treatment has been continued to the present time over periods of five, five, three and four months respectively. In no instance has there been any diminution of the ability of cortisone to suppress the output of 17-ketosteroids.

From the limited data cited above, it would seem that initial doses of 50 to 100 mg. of cortisone daily in older children or 25 to 50 mg. daily in young infants might be desirable to cause a rapid decrease of the 17-ketosteroid excretion. After this the suppression may be maintained by doses of 25 mg. daily, or 50 mg. every other day, in older children, whereas considerably smaller doses may be effective in young infants who have relatively lower pretreatment levels of 17-ketosteroids. It is probable that in Patient 8, an infant first treated at 11 weeks, the dose of 25 mg. of cortisone daily is excessive for prolonged use and is producing symptoms similar to Cushing's syndrome. It is obvious that further experience is necessary to determine the optimal maintenance dose and that clinical effects rather than steroid excretion must be considered in determining the therapeutic efficiency of cortisone.

The duration of the effect of cortisone after its withdrawal has been studied in only 1 patient (Case 1). After treatment with 100 mg. of corti-

sone daily for fifteen days, the excretion of 17-ketosteroids did not increase until ten days after its withdrawal and returned to the pretreatment level after about three weeks. Whether long-continued treatment will eventually result in inability of the adrenals to recover their former hyperactivity remains to be determined.

Effects of cortisone on excretion of biologically active androgen

The question arose as to whether the fall of urinary 17-ketosteroids represented a decreased excretion of adrenal androgen or was due to alteration in the excretion of nonandrogenic adrenal steroids. In 2 of our patients bioassays for urinary androgens were made before and during cortisone therapy by Dr. Ralph Dorfman, using the chick comb test, with the following results:

	Androgen assay i.u. per day (androsterone standard)		*17-Ketosteroids* mg. per day	
	Before treatment	*During treatment*	*Before treatment*	*During treatment*
Case 1	140	12.5	42	5
Case 3	150	14.4	48	6

The bioassays made in these cases show a reduction in the active androgen excreted to about one-twelfth of the pretreatment level whereas the 17-ketosteroids were reduced to only one-eighth.

In the patients over 2 years of age cortisone failed to reduce the 17-ketosteroid output below 3 to 6 mg. per day. Since these amounts are normal for adolescents but higher than those found in young children, we feared that they might be sufficient to cause some abnormal androgenic effects. Bioassays, however, suggest that a portion of the 17-ketosteroids consists of end-metabolites of the cortisone administered and is not active androgen. Sprague *et al.* (7) found that when cortisone was given to patients with rheumatoid arthritis who originally excreted normal amounts of 17-ketosteroids, the output decreases at first. On the other hand, in 1 patient who originally excreted 2 to 4 mg. of 17-ketosteroids per day the administration of 200 mg. of cortisone daily caused an increase to 14 mg. per day. These workers conclude that "the initial fall from normal levels indicates suppression of cortical function and that the 17-ketosteroids excreted during administration of cortisone are derived largely, if not entirely, from the cortisone administered." This conclusion is borne out by the fact that Thorn and his coworkers (8) found that in 8 female patients with Addison's disease 100 mg. of cortisone daily increased the excretion of 17-ketosteroids from a pretreatment level of 1.1 mg. per day to 2.5 mg. per day, and in 2 males from 4.1 mg. to 8.4 mg. per day.

Whether the relatively small amount of biologically active androgen as determined by bioassay is sufficient to cause early growth of sexual hair and progressive virilization during childhood can be determined only by therapeutic trial over long periods of time.

Effect of cortisone on excretion of estroids and biologically active estrogen

Before treatment the output of urinary estroids, determined chemically by Jailer's method, ranged from 38 to 105 micrograms per day except in Case 2, a boy of $2\frac{1}{2}$ years who excreted 24 micrograms per day and Case 8, a female pseudohermaphrodite of 7 weeks who excreted 23 micrograms per day. These values are high compared to normal females of comparable age. (In our laboratory we have found less than 10 micrograms per day in preadolescent girls, and 20 to 40 micrograms per day in adult females.) With cortisone therapy the excretion of estroids was affected in the same direction as that of 17-ketosteroids, decreasing under treatment and rising again on its withdrawal (Figs. 1–7). This suggests that at least a portion of these steroids is derived from the adrenal.

In 1 patient (Case 7) parallel chemical determinations of estroids and bioassays of estrogen by the method of Evans, Varney and Koch (9) were made with the following results:

	Before treatment	*During treatment*
Estroids (determined chemically)	93 micrograms per day (average of 4 days)	40 micrograms per day (18th day of treatment)
Estrogen (bioassay)	40 i.u. per day	4 i.u. per day (13th day of treatment)

The values of estrogen found by bioassay before treatment are considerably higher than normal for preadolescent girls and are comparable to those of mature women. The decrease in the output of biologically active estrogen is even greater than that of chemically determined estroids. The lack of female development in spite of this high secretion of estrogen and the action of cortisone in bringing about feminization will be discussed in a later section.

Effect of cortisone on excretion of corticosteroids

The effect of cortisone on the excretion of corticosteroids was somewhat irregular and difficult to interpret. The output of these steroids was higher than normal before treatment. Under therapy the excretion was generally reduced, although there were irregular fluctuations. In 1 patient (Fig. 1) treated with 100 mg. of cortisone daily, the excretion of corticosteroids decreased from levels of 1.36 to 1.74 mg. per day to 0.58 mg. on the first

day of treatment and remained between 0.37 and 0.51 mg. during the next eleven days. On the twelfth and fourteenth days of treatment the output rose to 1.04 and 1.07 mg. respectively. We believe that this may be due to the fact that a portion of the cortisone administered is excreted in the corticosteroid fraction of the urine. Sprague and his coworkers (7) found that in a patient with rheumatoid arthritis the urinary corticosteroids increased from approximately 1 mg. to 3 mg. after 100 mg. of cortisone had been given daily for two weeks but subsequently the output decreased. In another patient 100 mg. of cortisone daily had little effect on the output of corticosteroids, whereas 200 mg. increased the excretion to more than 5 mg. per day. Furthermore, these workers were able to isolate small amounts of cortisone from the urine of patients who were treated with cortisone. Rosemberg and Lewis (10) found that the urine of patients receiving cortisone contained increased amounts of eosinophil-reducing substance when assayed on adrenalectomized mice.

The irregularities of excretion of corticosteroids and the variations observed in different cases of congenital adrenal hyperplasia treated with cortisone might be explained by the fact that two factors affect their output—1) suppression of their secretion by the adrenal, leading to reduced urinary output, and 2) excretion in the urine of a portion of the cortisone administered.

Responsiveness of adrenals to stimuli during cortisone therapy

In our third patient (Fig. 3) the onset of a slight respiratory infection on the tenth day of cortisone treatment at a time when the 17-ketosteroid excretion had been reduced to 9 mg. per day resulted in a sharp rise in the output to 52 mg. per day and a corresponding increase in the urinary estroids and corticosteroids. It seems probable that 25 mg. of cortisone given daily was able to suppress the adrenal activity of this patient under the normal conditions of health, but that when more adrenocorticotropic hormone was secreted by the pituitary under the stress of an acute illness, the adrenal glands were still capable of responding with increased secretion. Later, on the forty-fifth day of treatment with cortisone, this patient's response was tested by giving 80 mg. of ACTH over a 24-hour period. At this time there was a relatively small response with a rise of urinary 17-ketosteroids from 6 mg. to 14 mg. a day. In order to determine whether continued treatment with cortisone might cause a progressive decrease in the ability of the adrenals to respond to stimuli, Patient 4 was tested with 85 and 100 mg. of ACTH before treatment and at various intervals after cortisone therapy was begun. As shown in Figure 4, ACTH had very little effect on the 17-ketosteroid excretion before treatment, although there was a more definite increase in the corticosteroids and estroids.

Diminished response to ACTH has been observed by Bartter, Forbes and Leaf (11) and by Thorn and Forsham (12) in cases of congenital adrenal hyperplasia, although in other patients with this disorder we have found considerable increase of the 17-ketosteroids after ACTH (13). Failure of response might be explained by assuming that the adrenals are already secreting at their maximum capacity and are incapable of increased activity. In our patient (Case 4) tests with ACTH, repeated on the seventeenth and thirty-second days of treatment while 25 mg. of cortisone were being given daily, caused moderate increases of steroid excretion of about the same magnitude. The dose of cortisone was increased to 50 mg. a day on the thirty-fourth day. A repetition of the ACTH test on the forty-seventh day resulted in a much smaller response. Further studies on patients under long-continued cortisone therapy are indicated, to determine whether the adrenals undergo progressive inability to respond to stimuli and eventually permanent atrophy. It has been shown by Stebbins (14) that the administration of 3 mg. of cortisone to rats over periods of ten to forty-two days caused marked atrophy of the inner zones of the adrenal cortex while the glomerulosa remained intact.

Effects of cortisone on protein and carbohydrate metabolism

Studies made on 4 of the patients (Cases 3, 4, 5 and 7) who were maintained on approximately constant diets showed that with the administration of cortisone there was increased excretion of urinary nitrogen accompanied in some instances by loss of weight, especially in the early stages of treatment. This should be regarded as a desirable effect since these patients when untreated exhibit more active protein anabolism than normal, leading to excessive growth and muscular development.

In the case studied no abnormalities in carbohydrate metabolism were observed.

Effects of cortisone on electrolyte regulation

Six of the patients had never shown any evidence of electrolyte disturbance and no change in the serum electrolytes was noted during treatment. In 1 of these patients (Fig. 5) there seemed to be a decrease in sodium excretion when 100 mg. of cortisone was being given daily and an increase to pretreatment level when the dose was decreased to 25 mg. daily.

The effects of cortisone were of special interest in the 4 patients who had shown severe deficiency of electrolyte regulation (Cases 6, 8, A and B). *Patient 6* had had pellets of desoxycorticosterone implanted six months prior to cortisone therapy. At the time of treatment he was receiving a high intake of NaCl and had normal concentrations of serum electrolytes.

The administration of 25 mg. of cortisone daily caused no change in these, nor any alteration in the excretion of sodium. However, a marked increase in the urinary sodium occurred the second to fourth day after cortisone was discontinued (Fig. 6). *Patient 8* was admitted to the hospital at the age of 7 weeks in a state of severe dehydration with serum sodium 130 mEq./L., potassium 9.0 mEq./L., chloride 112 mEq./L. and CO_2 18 mEq./L. With an average intake of 120 mEq. of sodium per day over four weeks, hydration was brought about and a gradual gain of weight occurred, although the abnormal pattern of electrolytes was not corrected. Additional treatment with 25 mg. of cortisone daily restored the serum electrolytes to approximately normal values, although unexplained fluctuations occurred (Fig. 8). In this young infant 25 mg. of cortisone daily seems to be too large a dose for continued administration because of the development of obesity and symptoms suggestive of Cushing's syndrome. Extensive balance studies and other investigations now in progress will be reported later. *Patient A*, treated by Dr. Faber and his associates, had had severe and nearly fatal Addisonian crises shortly after birth that were controlled with desoxycorticosterone by injection and later by pellet implants. In the four months during which he has received cortisone, no additional desoxycorticosterone has been required but it is not known whether the pellets were exhausted. *Patient B*, treated in Zurich, also had had Addisonian symptoms and was receiving 2.5 mg. of desoxycorticosterone and 3 Gm. of extra NaCl per day. The addition of 15 mg. of cortisone daily caused no change in the urinary electrolytes during a six-day period of treatment and for three days after its withdrawal.

Additional studies are necessary in cases of congenital adrenal hyperplasia of the salt-losing type to determine more definitely the effects of cortisone on the electrolytes and to decide whether treatment with desoxycorticosterone in addition to cortisone is desirable in some cases. Thorn and his coworkers (8) found that in Addison's disease 100 mg. of cortisone induced a retention of sodium and chloride with increased potassium excretion approximately equal to that of 2 mg. of desoxycorticosterone acetate. However, in some patients already receiving DCA the addition of cortisone caused increased excretion of sodium.

Effects of cortisone on eosinophils

Before treatment, eosinophil counts in this series of patients usually ranged from 100 to 200 per cu. mm. but in some cases they were as low as 75 and in others as high as 700. The effects of cortisone were not constant. In most cases there was a definite decrease but 1 patient receiving 100 mg. of cortisone (Case 1) and one receiving 50 mg. (Case 4) showed no fall in the eosinophil count.

Effects of cortisone on virilization

Although most of our patients have not been treated for sufficient time to evaluate the effects of cortisone on virilization, a number of observations suggest that there may be suppression. Patient 4, whose clitoris had been amputated some years previously, had a clitoral stump which was apparently in a constant state of painful erection and could be felt as a hard mass the size of the end of one's thumb. Within a week or ten days after beginning cortisone treatment the child stated voluntarily that the pain had stopped. The stump of the clitoris could no longer be palpated and no more erections were detected during the forty-eight days of treatment. In a number of cases (Cases 3, 5 and 7) there was decrease of acne and seborrhea. In Patient 7 an attempt to measure the rate of growth of axillary hair (by weighing the hair shaved at regular intervals) has not been very successful, although after three or four months of treatment there has apparently been some decrease. In the 2 patients who have now been treated for about five months (Cases 5 and 7), the development of secondary female characteristics described in the next section strongly suggests that virilization has been suppressed.

Feminization under cortisone therapy

Patient 5, aged $8\frac{1}{2}$ years, before treatment had infantile nipples and no mammary tissue. After receiving 25 mg. of cortisone daily for about a month, swelling of the breasts was noted for the first time. They became painful and grew progressively larger. When she was examined on the one hundred and fiftieth day of treatment the nipples were erectile and the glandular structure measured 8 cm. in diameter. The vaginal smear showed moderate estrogenic changes.

In patient 7, aged $18\frac{1}{2}$ years, the breasts had failed to develop spontaneously. Mammary development induced by stilbestrol at the age of 9 to 11 years had disappeared completely at the time of cortisone treatment, although the nipples were fairly large and the areolae were deeply pigmented, as was the rest of her body. Within a month or two after beginning cortisone there was definite development of the breasts which have now attained a diameter of 7 cm. Prior to cortisone slight menstrual staining had occurred only twice during the time she was receiving stilbestrol in 1941–1943. On the thirty-third day after beginning cortisone, menstruation began. There have now been four painless menstrual periods at intervals of twenty-nine to thirty-one days with fairly abundant flow lasting three to four days. Vaginal smears taken during the third and fourth cycles have shown marked estrogenic changes.

Since untreated female pseudohermaphrodites excrete biologically active estrogens, presumably elaborated by the adrenals, in as large amounts as

do normal women, the absence of female development is probably due to the antagonistic effects of the excessive amounts of androgen secreted. That such an antagonism between estrogen and androgen exists has been shown by Shorr and Carter (15) who found that it was necessary to give as much as 30 mg. of estradiol benzoate daily to one of their patients with female pseudohermaphrodism before any changes in the vaginal smear occurred.

The rapid development of the female sex organs after instituting cortisone therapy probably is not due to a direct action of cortisone on the end-organs, since this has never been observed in children treated with cortisone for other conditions. It seems probable that before treatment the high concentrations of circulating adrenal estrogen and androgen inhibit the secretion of follicle-stimulating hormone by the pituitary, thereby preventing the maturation of the ovaries. The administration of cortisone, by suppressing the adrenal secretion of estrogen as well as androgen, may permit the secretion of pituitary gonadotropin, setting the normal pituitary-ovarian mechanism into action. Further studies are being made of the urinary gonadotropins and estrogens before and during cortisone therapy in order to investigate this hypothesis.

Effect of cortisone on blood pressure

Only recently have we been aware that hypertension occurs in some cases of congenital hyperplasia of the adrenals of the androgenic type. Two of our patients (Cases 2 and 7) have shown hypertension.

The effects of cortisone on the blood pressure could not be observed satisfactorily in Case 2 because its administration was followed by excitability which probably caused the blood pressure to rise. The hypertension was of such a degree that treatment was discontinued. Furthermore, this patient has vascular and cardiac changes.

Patient 7 (Fig. 7) had had hypertension for a long period. During a control period in the hospital there was no tendency for the pressure to decrease. Under cortisone therapy without other change of regimen, the pressure dropped to normal within ten days and there has been no tendency for it to rise again during five months of treatment, even though the patient has returned to normal life. This suggests that the hypertension in this case may have been related to abnormal hormones of the adrenal which were suppressed by cortisone.

Effect of cortisone on skin pigmentation

Only 1 of our patients (Case 7) has shown marked pigmentation of skin and gums resembling that of Addison's disease. All observers agree that

the pigmentation of the skin decreased definitely during the five months she has been treated with cortisone.

Toxic and unfavorable metabolic effects

Toxic or unfavorable effects of cortisone were encountered in only 2 cases. One was the mentally retarded 2½-year-old boy (Case 2) who became wildly excited and manic after receiving 50 mg. of cortisone daily for eight days. The other was the small infant (Case 8) whose face became round and full after receiving 25 mg. of cortisone daily for several months. We believe that smaller doses of cortisone would suffice to suppress her adrenal activity but wished to study the effects of 25 mg. doses on her electrolyte metabolism.

Mechanism of action of cortisone

To determine whether cortisone acts directly upon the adrenal glands or suppresses the secretion of pituitary adrenocorticotropin, Lewis and Rosemberg (16) administered 1.25 mg. of cortisone daily for twenty days to normal rats and to hypophysectomized rats treated with ACTH. In normal rats cortisone caused reduction in the weights of the adrenals; in hypophysectomized rats maintained on ACTH there was no effect. Although this suggests that the action of cortisone in the cases studied is through the pituitary, it does not imply that congenital adrenal hyperplasia is due to excessive secretion of adrenocorticotropin by the pituitary rather than to a primary disorder of the adrenal glands. Even though a primary disorder of the adrenal is responsible for the secretion of abnormal mixtures of steroids in large amounts, nevertheless it seems probable that the secretory activity of the pathologic adrenal glands is dependent upon the tropic function of the pituitary. We suggest that the action of cortisone is to suppress the output of pituitary adrenocorticotropin, thereby causing marked diminution of the secretion of the pathologic adrenals. With most of the activity of the abnormal adrenals suppressed, the normal requirement of the body for glycogenetic hormones is fulfilled by cortisone. If excessive amounts are administered, symptoms similar to Cushing's syndrome may occur.

It is not certain whether the suppression of adrenocorticotropin by cortisone impairs the electrolyte-regulating activity of the adrenals. The experiments on animals made by Deane and Greep (17), Jones (18) and Stebbins (14) suggest that the zona glomerulosa which supposedly secretes electrolyte-regulating hormone may function relatively independently of the pituitary. Furthermore, electrolyte disturbances usually do not occur in patients with panhypopituitarism. If this is the case, one might expect that the administration of cortisone will not impair electrolyte regulation

in those patients with congenital adrenal hyperplasia who do not have already a defect of this function.

SUMMARY

1. In the 10 cases of congenital adrenal hyperplasia studied, initial doses of 50 to 100 mg. of cortisone daily in older children, or 25 to 50 mg. daily in young infants, regularly caused rapid decrease of the urinary 17-ketosteroids to levels of 4 to 8 mg. per day in the older group, and to levels of 0.3 to 1.4 mg. per day in the younger group. The suppression of 17-ketosteroid excretion was subsequently maintained by the administration of 25 mg. of cortisone daily, or 50 mg. every other day, in older children; whereas doses varying from 10 to 25 mg. per day sufficed in the infants. The effects lasted as long as treatment was continued, the longest period in our series being five to six months.

2. In the 2 cases in which assays were made, the amounts of biologically active androgen in the urine were reduced to probably a greater degree than the 17-ketosteroids.

3. In all cases studied, the urinary estroids determined chemically were high before treatment and were reduced concomitantly with the 17-ketosteroids. In 1 case it was shown that biologically active estrogen followed the same pattern.

4. The corticosteroids of the urine were affected less regularly. Although there was generally a moderate decrease, in 1 case the output rose again slightly while treatment was being continued, possibly because a portion of these steroids may be derived from the cortisone administered.

5. In 1 case an acute infection occurring in the early stages of treatment with 25 mg. of cortisone daily caused a marked temporary increase in the urinary 17-ketosteroids, corticosteroids and estroids. The response of the adrenal to stimulation with ACTH was tested in 1 case before treatment was begun and at various stages during treatment; but more work is required to determine whether continuous treatment leads to progressive decrease in the reactivity of the adrenal.

6. No changes in carbohydrate metabolism were observed.

7. There was an increase in urinary nitrogen excretion suggesting decrease of protein anabolism.

8. In patients with congenital adrenal hyperplasia and defective electrolyte regulation, cortisone probably has some sodium-retaining activity. At this time it is not possible to decide whether desoxycorticosterone in addition to cortisone is required to maintain electrolyte equilibrium in some of these patients.

9. In most cases the period of treatment has been too short to note definite decrease of virilization. In 1 patient erections of the stump of the

clitoris stopped promptly. There has been some decrease of acne and seborrhea in a number of cases. In 2 patients treated for five months, development of the breasts and estrogenic changes of the vaginal smear have occurred and 1 girl has now had four regular menstrual periods.

10. In 1 patient hypertension disappeared under treatment.

11. In the patient showing Addisonian-like pigmentation there was marked fading.

12. Treatment with cortisone in small doses offers the possibility of preventing or suppressing the progressive virilization which occurs with congenital adrenal hyperplasia and may permit the normal development of feminine characteristics in female pseudohermaphrodites. A great deal more work, however, is required to evaluate adequately such therapy and determine the optimum dosage and route of administration.

Acknowledgment

We wish to acknowledge the faithful assistance of Mr. Henry M. Schulte, Jr., and Mrs. Mary Ellen Crafton in performing steroid determinations.

The ACTH was kindly furnished by Dr. John R. Mote, The Armour Laboratories, Chicago, Illinois.

REFERENCES

1. Wilkins, L.: Hyperadrenocorticism, *Pediatrics* **3**: 533, 1949.
2. Wilkins, L.; Lewis, R. A.; Klein, R., and Rosemberg, E.: The suppression of androgen secretion by cortisone in a case of congenital adrenal hyperplasia, *Bull. Johns Hopkins Hosp.* **86**: 249, 1950.
3. Wilkins, L.; Lewis, R. A.; Klein, R., and Rosemberg, E.: Die Wirkung von Cortisone auf die Ausscheidung der 17-Ketosteroide und anderer Steroide bei Patienten mit kongenitaler Niebennierenhyperplasie, *Helvet. paediat. acta* **5**: 418, 1950.
4. Jailer, J. W.: A fluorometric method for the clinical determination of estrone and estradiol, *J. Clin. Endocrinol.* **8**: 564, 1948.
5. Heard, R. D. H., and Sobel, H.: A colorimetric method for the estimation of reducing steroids, *J. Biol. Chem.* **165**: 687, 1946.
6. Talbot, N.; Saltzman, A. H.; Wixom, R. L., and Wolfe, J. K.: The colorimetric assay of urinary corticosteroid-like substances, *J. Biol. Chem.* **160**: 535, 1945.
7. Sprague, R. G.; Power, M. H.; Mason, H. L.; Albert, A.; Mathieson, D. R.; Hench, P. S.; Kendall, E. C.; Slocumb, C. H., and Polley, H. F.: Observations on the physiologic effects of cortisone and ACTH in man, *Arch. Int. Med.* **8**: 199, 1950.
8. Thorn, G. W.; Forsham, P. H.; Bennett, L. L.; Roche, M.; Reiss, R. S.; Slessor, A.; Flink, E. B., and Somerville, W.: Clinical and metabolic changes in Addison's disease following the administration of compound E acetate, *Tr. A. Am. Physicians* **62**: 233, 1949.
9. Evans, J. S.; Varney, R. F., and Koch, F. C.: The mouse uterine weight method for the assay of estrogens, *Endocrinology* **28**: 747, 1941.
10. Rosemberg, E., and Lewis, R. A.: Reduction in eosinophil level of adrenalectomized

mice following injection of adrenocorticotropin, 11-dehydro-17-hydroxy-corticosterone and lipid extracts of human urine, *J. Applied Physiol.* **3**: 164, 1950.

11. Bartter, F. C.; Forbes, A. P., and Leaf, A.: Congenital adrenal hyperplasia associated with the adrenogenital syndrome: an attempt to correct its disordered hormonal pattern, (abstract) *J. Clin. Investigation* **29**: 797, 1950.
12. Thorn, G. W., and Forsham, P. H.: Metabolic changes in man following adrenal and pituitary hormone administration, *in* Recent Progress in Hormone Research, New York, Academic Press Inc., 1949, vol. 4, p. 262.
13. Wilkins, L.; Klein, R., and Lewis, R. A.: The response to ACTH in various types of adrenal hyperplasia. Proceedings of the First Clinical ACTH Conference, Philadelphia, The Blakiston Co., 1950, p. 184.
14. Stebbins, R. B.: Cytochemical changes in the adrenal cortex of the rat following the administration of cortisone, *Fed. Proc.* **9**: 345, 1950.
15. Shorr, E., and Carter, A. C.: A study of the biological activity and the magnitude of endogenous androgen production in a case of adrenogenital syndrome. Transactions of the 16th Conference on Metabolic Aspects of Convalescence, New York, Oct. 1947. Publication of Josiah Macy Jr. Foundation, New York, pp. 132–145; also Carter, A. C., and Shorr, E.: *J. Clin. Endocrinol.* **8**: 583, 1948.
16. Lewis, R. A., and Rosemberg, E.: The effects 17-hydroxy-11-dehydrocorticosterone upon the adrenals of normal rats and of hypophysectomized rats maintained with adrenocorticotropin, *Endocrinology* (in press).
17. Deane, H. W., and Greep, R. O.: A morphological and histochemical study of the rat's adrenal cortex after hypophysectomy with comments on the liver, *Am. J. Anat.* **79**: 117, 1946.
18. Jones, I. C.: The relationship of the mouse adrenal cortex to the pituitary, *Endocrinology* **45**: 514, 1949.
19. Prader, A.: Pseudohermaphroditismus femininus mit kongenitaler Nebennierenrinden-Insuffizienz. Günstige Wirkung von Cortison, Desoxycorticosteronacetat und Kochsalz, *Helvet. paediat. acta* **5**: 426, 1950.

STUDIES OF URINARY CORTICOSTEROID BY A METHOD PERMITTING ANALYSIS OF STEROIDS POORLY SOLUBLE IN WATER

II. INSUFFICIENT ADRENAL FUNCTION*

CHARLES W. LLOYD, M.D. AND JULIA LOBOTSKY, M.S.

From the Departments of Obstetrics, Medicine, and Physiological Chemistry, Syracuse University College of Medicine, Syracuse, N. Y.

A METHOD for determination of urinary corticosteroid permitting estimation of those steroids poorly soluble in water as well as those which are freely soluble in water has been described (1). The corticosteroid estimated by this method has been termed the "total corticosteroid." At times the steroids which partition from benzene to water have also been determined. The material which partitions freely from benzene into water is called the "water soluble corticosteroid." The material which remains in the benzene fraction is called the "corticosteroid poorly soluble in water." The excretion of the materials by subjects with normal adrenal function has been described (1). The present report concerns the excretion of these materials by patients with adrenal insufficiency.

METHODS

The methods of urine collection and analysis have been previously described (1).

RESULTS

Total corticosteroid excretion

Eleven patients with adrenal insufficiency have been studied. Single or infrequent corticosteroid measurements were carried out on 7 of these patients. These values and pertinent clinical data are shown in Table 1. Each of these patients had the classic picture of Addison's disease with a history of weakness, weight loss, hypotension, gastro-intestinal disturbances and pigmentation. Most of the patients had been in crisis at least once. Each had low 17-ketosteroid excretion. Other diagnostic procedures such as the Robinson-Power-Kepler test, insulin tolerance and eosinophil response to epinephrine were performed in those patients

Received for publication June 6, 1950.

* This work was in part supported by grants from the Schering Corporation and from the Hendricks Research Fund of Syracuse University College of Medicine.

in whom the slightest question of the diagnosis seemed possible. The results were positive whenever these tests were made.

In Figure 1 are shown the corticosteroid values of Patient 8, a 24-year-old woman with Addison's disease. On the first day of the study, the corticosteroid excretion was high as a result of the large amount of cortical steroids given in treatment of a crisis. Following this day, the corticosteroid

TABLE 1. CORTICOSTEROID EXCRETION BY PATIENTS WITH ADRENAL INSUFFICIENCY

Subject	Sex	Age (yrs.)	Total corticosteroid, γ of formaldehyde per 24 hrs.	Condition at time of urine collection	Clinical history
1-M.W.	F	44	6.4	Before therapy	Idiopathic insufficiency for 6 months.
			11.0	Pellets of DCA	
2-R.G.	M	37	14.3	Pellets exhausted	Idiopathic insufficiency for 5 years, treated with DCA pellets.
			9.0	Signs of insufficiency-given 50 mg. of DCA crystals	
			11.3	3 days after DCA (crystal) injection	
			15.0	6 days after DCA (crystal) injection	
			16.4	9 days after DCA (crystal) injection	
3-H.L.	M	45	15.3	DCA pellets-fair control	Idiopathic insufficiency for 1 year.
4-S.M.	M	48	22.4	DCA pellets-good control	Probably tuberculous adrenal insufficiency. Died of pulmonary tuberculosis.
5-B.B.	F	44	165.0	Crisis-large amounts cortical extract and DCA	Idiopathic adrenal insufficiency for 5 years, inadequately treated.
			150.0	Crisis-large amounts cortical extract and DCA	
			317.0	Overdose of DCA-55 mg.	
			52.0	48 to 72 hours after DCA overdose	
			36.5	72 to 96 hours after DCA overdose	
6-W.	F	45	22.2	No therapy for 4 days	Tuberculous peritonitis and adrenal insufficiency, relieved by DCA. Therapy omitted by error; patient died at end of collection period.
7-M.CH.	M	52	30.0	Pellets exhausted	Idiopathic insufficiency for 10 years, inadequately treated.

excretion rate fell to a lower level for several days and then desoxycorticosterone acetate (DCA) therapy caused a gradual climb to relatively high values by the forty-eighth day. At this time clinical overdosage phenomena became manifest. Periods of withdrawal of therapy beginning on the forty-ninth and sixty-seventh days on each occasion resulted in the appearance of low excretory rates and of signs of adrenal insufficiency on the fifth day without treatment. Following implantation of pellets there were several days when the rate of excretion was high. By the end of two weeks after the implantation of the pellets the excretory rate had again

dropped to a level as low as that found during insufficiency, even though the patient was clinically well.

Patient 8, (J. S.), was a 24-year-old woman who had had Addison's disease for four years, and who had had pellets of DCA implanted eighteen months before this study was begun. She had a history of at least three previous episodes of crisis and coma. During the night of the first day of the study, the patient was admitted to the hospital with hypotension and vomiting. On day 1 of the study, therapy consisted of parenteral saline and glucose solutions as well as 10 cc. intravenously and 20 cc. intramuscularly of aqueous Adrenal Cortical Extract (Upjohn) and 5 mg. of DCA (Ciba). Day 2 of the study, which was the first 24-hour period during which the urine was collected, began

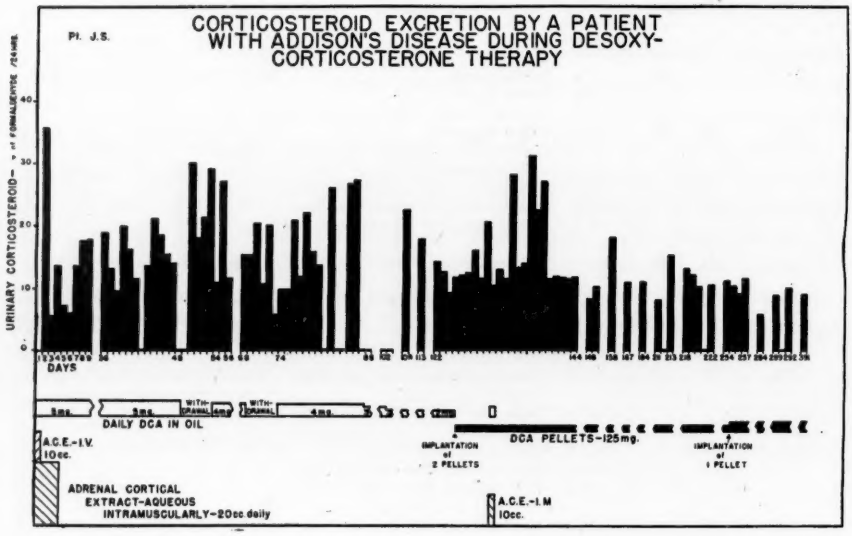

FIG. 1. Corticosteroid excretion of a 24-year-old woman with adrenal insufficiency. In this and in subsequent figures the bars represent total corticosteroid excretion.

twelve hours after admission. During each of days 2, 3, and 4 the patient received 20 cc. of aqueous adrenal cortical extract intramuscularly. Throughout the entire study, DCA was given either by daily intramuscular injections of oil solution or by pellet, except for the five-day periods beginning on days 48 and 68 respectively, when therapy was intentionally withheld until signs of insufficiency developed.

The patient responded rapidly to treatment and was asymptomatic by the second day of hospitalization. By the forty-eighth day, clinical evidence of slight overdosage was seen. A period of withdrawal began with the last dose of DCA on that day. Determinations of corticosteroid could not be made on the forty-eighth and forty-ninth days. Daily administration of 4 mg. of DCA in oil was commenced on the fifty-fourth day. Another period of withdrawal, beginning on the sixty-eighth day, again resulted in low corticosteroid excretion and development of signs of insufficiency on the fifth day after

withdrawal. Further administration of 4 mg. of DCA daily beginning on the seventy-fourth day resulted in signs of overdosage and a high excretory rate by the eighty-sixth day of the study. Dosage of DCA was decreased until on the 103rd day a dose of 2 mg. of DCA daily was begun. This dose was judged clinically to be optimal. It resulted in relatively low urinary corticosteroid excretion.

On the 125th day 2 DCA pellets (125 mg. each) were implanted subcutaneously. No further therapy was given except for single injections of 5 mg. of DCA and 10 cc. of aqueous Adrenal Cortical Extract on the 131st day when the patient had a 24-hour long gastro-intestinal disturbance, apparently infectious in origin. Thereafter, the patient was in good clinical condition until the 255th day, when slight weakness and easy fatigability warranted the implantation of a third pellet. It was impossible to collect urine sufficiently long after this implantation to determine if a period of high excretion occurred. Corticosteroid levels at intervals thereafter have all been in the low range, although no evidences of insufficient treatment have been detectable.

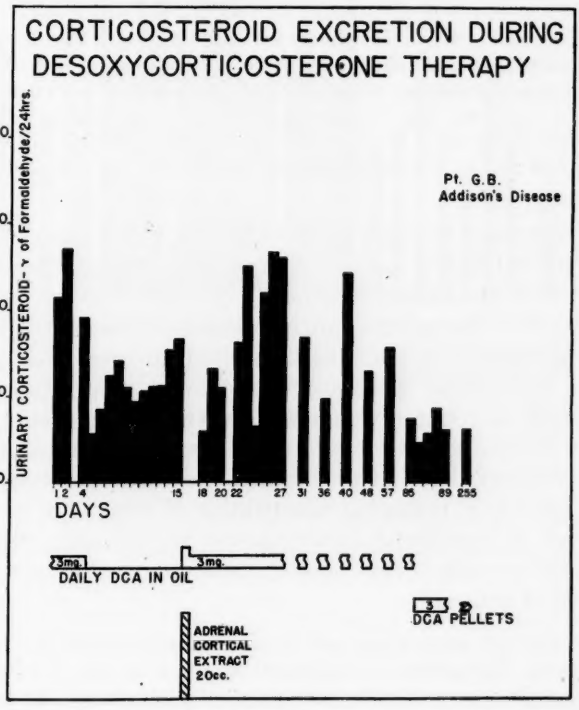

FIG. 2. Corticosteroid excretion of a 27-year-old man with adrenal insufficiency.

Figure 2 represents the corticosteroid excretion of Patient 9, a 27-year-old man with Addison's disease. At the beginning of the study, while the patient was receiving 3 mg. of DCA daily, the corticosteroid excretion was fairly high. A period of withdrawal resulted in a low rate of excretion

with the lowest level at the beginning of the withdrawal period and a tendency for the excretion values to be slightly higher at the end of the withdrawal period. Therapy again resulted in an increased excretion of corticosteroid until a relatively high level was attained. As therapy became stabilized, the excretory rate decreased. Implantation of pellets was followed by a very low corticosteroid excretion although the patient was in good clinical condition. The wide daily fluctuations seen when DCA in oil was given by injection were not seen after pellet implantation.

Patient 9, (G. B.), was a 27-year-old man who had had known Addison's disease for eighteen months and a history of one episode of crisis. Therapy before the onset of the study had consisted of intramuscular injections of DCA in oil, usually 3 mg. daily, although occasionally the dose was omitted for one or two days. At the time the study was begun, the patient's disease was well controlled.

A period of withdrawal of DCA began on the fifth day of the study. The patient felt well until the eighth day of the withdrawal period, at which time he complained of slight weakness and dizziness. On the twelfth day without therapy he had an episode of weakness, nausea and vomiting, although blood pressure was maintained at its usual level. Treatment again was begun on that day, with 20 cc. of aqueous Adrenal Cortical Extract intramuscularly being given in addition to 5 mg. of DCA in oil. Three 125 mg. pellets were implanted on the eighty-sixth day. One pellet was extruded on the 250th day.

Figure 3 represents the corticosteroid excretion of Patient 10, a 27-year-old man with Addison's disease. At the beginning of the study, when the patient had insufficient treatment, corticosteroid excretion was at the lower limit of the normal range. Administration of DCA caused no definitely significant increase in corticosteroid (average 23.2γ per twenty-four hours). Withdrawal of therapy was followed by a statistically significant increase in excretion (average 29γ per twenty-four hours, $p = <.01$). A tendency for the excretory rate to be lower is apparent towards the end of the withdrawal period when the patient was beginning to manifest signs of insufficiency. Following reinstitution of treatment, a short period of fairly high corticosteroid excretion was found, after which a lower excretory rate prevailed. One high corticosteroid value was found after implantation of pellets.

Patient 10, (M. C.), was a 27-year-old male with adrenal insufficiency which was of moderate severity. This patient had pigmentation, and symptoms of weight loss, weakness and fatigue. He was unable to achieve a normal water diuresis. He was sensitive to insulin and 17-ketosteroid excretion was 2.5 mg. in twenty-four hours. Roentgenograms of the chest showed a pulmonary infiltration interpreted as being tuberculous. During bed rest without cortical steroid therapy, he felt weak but adrenal insufficiency never progressed to the point of crisis. Urinary corticosteroid values in the fall of 1947, approximately fourteen months before the beginning of the study shown in Figure 3, were in the lower range of normal. It was felt at that time that this patient had considerable

cortical function but that the amount of steroid available was inadequate to supply the demand, which probably was increased because of his pulmonary infection. Five 125 mg. pellets of DCA were implanted subcutaneously fourteen months before the beginning of the study in Figure 3.

At the beginning of the study, the patient's pellets were not palpable and he had signs

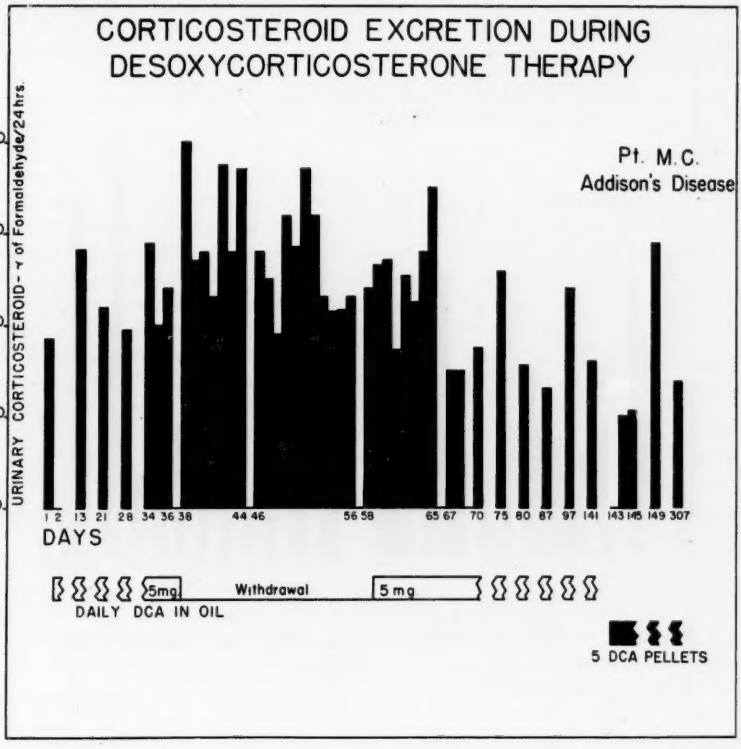

FIG. 3. Corticosteroid excretion of a 27-year-old man with adrenal insufficiency.

of cortical insufficiency. DCA in oil, 5 mg. daily, was administered beginning on day 2. The patient's clinical status improved within two days and by the fifth day after beginning daily DCA injections, he was in excellent clinical condition. A period of withdrawal of DCA was begun on the thirty-eighth day of the study. During the first sixteen days of the withdrawal period the patient felt well; in fact, better than while he was receiving DCA. Thereafter for the next five days the patient felt progressively weaker and more tired until it became necessary to resume treatment on the twenty-second day following withdrawal of therapy. After the termination of the withdrawal period, therapy consisted of the daily administration of 5 mg. of DCA in oil. Implantation of 5 DCA pellets, 125 mg. each, was carried out on the 143rd day of the study.

In Figure 4 are shown the corticosteroid values of Patient 11. This patient excreted a relatively large amount of corticosteroid at the beginning of the study while she was receiving small daily doses of aqueous extract and was on a high salt, low potassium diet. Withdrawal of all therapy was followed by lower levels of urinary corticosteroid and administration of DCA resulted in relatively little, if any, increase in corti-

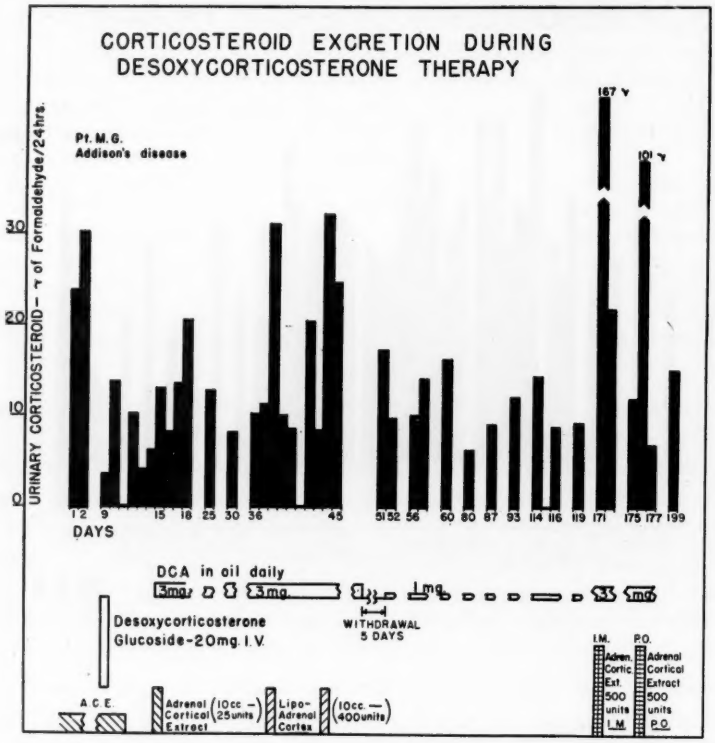

FIG. 4. Corticosteroid excretion of a 26-year-old woman with adrenal insufficiency.

costeroid. Administration of adrenal cortical extract on three occasions intramuscularly and once orally resulted in large increases in corticosteroid output. These increases persisted for only one or two days.

Patient 11, (M. G.), was a 26-year-old, single woman with adrenal insufficiency. This patient had the onset of her illness five years before with weakness, fatigue, weight loss, nausea and vomiting, pigmentation, and hypotension. A diagnosis of Addison's disease was made at another hospital based on the history, positive results with the Robinson-Power-Kepler water test, and development of signs of acute insufficiency following three days of salt restriction. Therapy before the beginning of this study had consisted of a

high salt, low potassium (Harrop) diet and daily injections of from 2 to 10 cc. of aqueous Adrenal Cortical Extract (Upjohn). When the present study was begun, the patient complained of poor strength and of frequent episodes of nausea and vomiting.

During the control period, treatment consisted of 4 cc. daily of aqueous Adrenal Cortical Extract (Upjohn). On day 9 of the study, desoxycorticosterone glucoside (Ciba), 20 mg., was given intravenously. A prompt subjective improvement lasting two days occurred. No further desoxycorticosterone was given for five days and no further aqueous Adrenal Cortical Extract for four days. On the sixth day after injection of the desoxycorticosterone glucoside, the patient's weakness necessitated administration of 10 cc. of Adrenal Cortical Extract and the commencement of daily injections of 3 mg. of DCA in oil. Beginning at this time the patient was given an unrestricted diet with salt intake of approximately 8 Gm. daily. On the second day after beginning of the DCA therapy the patient's weakness had disappeared and her general condition was much improved. After one week of daily injections of 3 mg. of DCA, edema developed in spite of continued hypotension (B.P. 86/65). The dose of DCA was then decreased to 2 mg. daily, but edema persisted for one week. At this time serum sodium was 146 mEq./L. The dosage of DCA was maintained at the same level (2 mg. per day) and the patient was given intramuscularly 10 cc. of Lipo-Adrenal Cortex. A prompt diuresis occurred, beginning about four hours after injection, continuing for twenty-four hours and causing a 2-pound weight loss and a twofold increase in urine volume. The continued administration of 2 mg. of DCA daily again resulted in edema and on the forty-fourth day the patient was again given 10 cc. of Lipo-Adrenal Cortex. This injection was also followed by a brisk diuresis with a 5-pound weight loss.

On the forty-seventh day, a period of withdrawal of DCA therapy was again attempted. This period lasted for five days and was terminated because of the patient's weakness and nervousness. A maintenance dose of 1 mg. daily of DCA was given from day 52 to day 162. On the 166th day the DCA dose was increased to 3 mg. daily in order to produce edema. On this day her weight was 104.5 pounds. This dose was maintained for eleven days. By the fourth day of the extra DCA therapy the patient had gained to 108 pounds and was clinically edematous. Urine volumes during this period were all less than 850 cc. in twenty-four hours. The patient was given, intramuscularly, 500 units of adrenal cortical extract (hog) in 2 cc. of propylene glycol (Upjohn). A marked diuresis occurred which continued for twenty-four hours and resulted in the loss of 2 pounds of weight and in an increase in urine volume to 2,255 cc. in twenty-four hours. The daily dose of 3 mg. of DCA was continued and urine volumes remained less than 850 cc. in twenty-four hours. On the 176th day, following a weight gain to 112 pounds and the appearance of edema, the patient was given 500 units of the same adrenal cortical extract orally. Diuresis occurred with a weight loss of 4 pounds and an increase in urine volume to 2,620 cc. in twenty-four hours.

Figure 5 summarizes the twenty-four-hour total corticosteroid excretion values for normal subjects and the patients with adrenal insufficiency described in this study. No difference exists between the values found for normal males and females. The subjects with adrenal insufficiency who were in an untreated state, in other words who had never had adrenal cortical steroid therapy or whose treatment had been interrupted for a sufficient period of time to develop clear cut signs of insufficiency, had corticosteroid excretion values below the lower range of normal. Patients

with adrenal insufficiency who received large doses either of DCA or adrenal cortical extract were able to excrete large amounts of corticosteroid. Following implantation of pellets, excretion was at a low level, usually below the normal range. The high values in this group are those obtained immediately after implantation of pellets when a transitory high level of absorption was present.

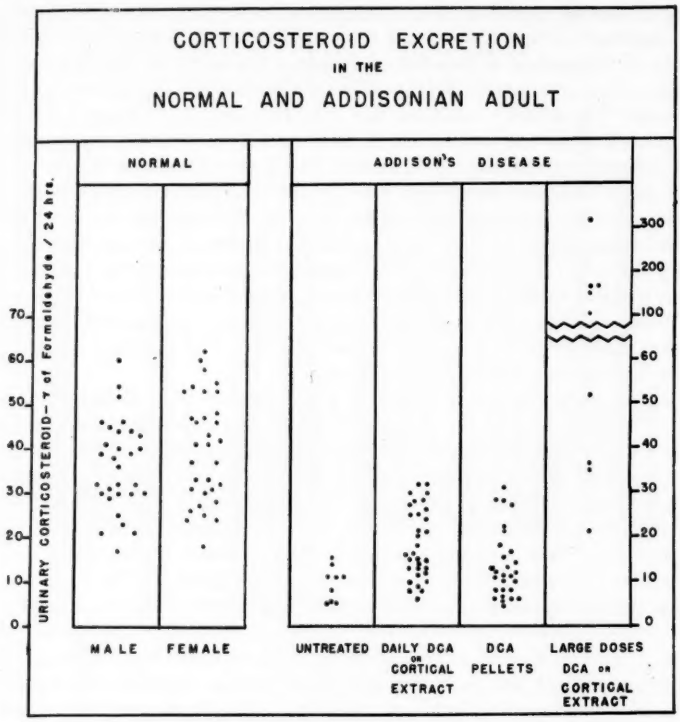

FIG. 5. Summary of corticosteroid excretion values of normal adults and of those with adrenal insufficiency.

Comparison of total and water-soluble corticosteroid of patients with adrenal insufficiency

Simultaneous determinations of total and water-soluble corticosteroid have been made on 26 twenty-four-hour urine specimens collected from 8 patients with adrenal insufficiency. These values are shown in Table 2. The total corticosteroid values have also been included in the data illustrated in Figures 1 to 4 or listed in Table 1. When no therapy was given, when the dose of DCA was not excessive or when treatment consisted of

TABLE 2. TOTAL AND WATER-SOLUBLE CORTICOSTEROID EXCRETION BY PATIENTS WITH ADRENAL INSUFFICIENCY

Subject	Total corticosteroid, γ of formaldehyde per 24 hrs.	Water-soluble corticosteroid, γ of formaldehyde per 24 hrs.
1-M.W.	6.4	6.2
2-R.G.	15.0	14.0
	14.3	15.1
	11.3	11.1
	16.4	11.3
3-H.L.	15.0	18.0
5-B.B.	317.0	6.0
8-J.S.	10.3	7.0
	11.4	13.6
	5.6	6.2
	8.7	8.4
	6.4	11.2
	9.8	11.8
9-G.B.	11.3	8.4
	16.1	10.7
10-M.C.	24.0	20.0
11-M.G.	24.0	18.0
	31.0	34.0
	8.5	12.0
	20.0	16.0
	32.0	28.0
	25.0	29.0
	17.0	13.6
	14.0	18.0
	16.0	15.6
	15.0	18.0

whole adrenal cortical extract, essentially all of the corticosteroid was water-soluble. However, when the dose of DCA was excessive (B.B.), a very large amount of the injected steroid was recovered in the urine and the major portion of this urinary steroid was poorly soluble in water. These data demonstrate that during its passage through the body, desoxycorticosterone is changed to a water-soluble material unless the amount

of material is excessively large. In this case, much less of the material is destroyed and much less of it is converted to a water-soluble form.

DISCUSSION

Subjects in whom adrenal insufficiency develops while not under stress can be classified in two groups according to corticosteroid excretion. One type of patient (exemplified by Patients 1, 2, 3, 4, 5 in Table 1 and by Patients 8 and 9 whose excretion data are shown in Figures 1 and 2) has relatively little if any endogenous adrenal steroid excretion. This probably is the result of inadequate hormone production because without replacement therapy insufficiency develops relatively quickly in these patients, and corticosteroid excretion reaches a low level. It is only when substitution therapy is given that they excrete amounts of corticosteroid approaching the normal range.

In the second group, patients with adrenal insufficiency have more adrenal cortical function. Patients 7, 10 and 11 (Figs. 3 and 4), fall into this category. In the untreated state this type of patient excretes an appreciable amount of corticosteroid and is able to continue in good health without therapy for a considerable length of time. However, continued omission of treatment eventually results in the development of severe insufficiency. Concomitantly with the appearance of the severe clinical insufficiency, urinary corticosteroid usually falls to a low level. These patients have a milder form of adrenal insufficiency and appear to represent individuals in whom the supply of adrenal steroid is not able to meet the demand, although the supply may appear to be quite large as judged by urinary excretion. Patients such as No. 6-W, Table 1, who died with signs of adrenal insufficiency in the presence of an overwhelming stress, may have high levels of corticosteroid excretion and presumably of cortical function, even though this function is not sufficient to meet the need for additional hormone resulting from the stress. This type of patient demonstrates that the urinary steroid excretion does not necessarily offer a good index of the state of competence of the adrenal cortex or of the adequacy of the supply of hormones for the body's demands.

Some of these data suggest that DCA inhibits endogenous steroid formation. Administration of DCA to M.C. (Fig. 3) resulted in comparatively little increase in corticosteroid excretion, particularly when the increment is compared with the corticosteroid increase which occurred following administration of DCA to patient J.S. (Fig. 1). Withdrawal of DCA from M.C. was followed by a relatively high rate of excretion for a few days with a subsequent lower rate of excretion as symptoms of insufficiency developed. This study suggests that the administration of DCA to this patient inhibited endogenous adrenal steroid production so that when this inhibition was removed, a temporarily greater rate of

steroid formation resulted. A similar, though much less marked, rise in corticosteroid excretion occurred on the two occasions when DCA was withdrawn from patient J.S. (Fig. 1). No explanation is available for the finding of a rapid drop in corticosteroid excretion after withdrawal of desoxycorticosterone therapy in a normal subject such as S.S. (reported elsewhere (1)) or in a patient with Addison's disease such as G.B. (Fig. 2), whereas in M.C. (Fig. 3) discontinuance of desoxycorticosterone caused an increase in corticosteroid. Differing rates of ACTH secretion or of recovery from the secretory inhibition caused by the DCA most probably are responsible for these different responses.

The widest day-to-day fluctuations in corticosteroid excretion occur in the normal individual or in patients with Addison's disease who fall in the second category described above, during periods without treatment or during the initial phases of therapy. A much more constant rate of excretion is present after good control has been attained in the patient with Addison's disease. Implantation of pellets is followed by a relatively high rate of excretion for a few days, probably because of increased absorption due to irregularities of the surface of the pellets or due to the absence of the fibrous tissue capsule which is present at a later date and which probably produces a lower absorption rate. After this peak excretion, a low and quite constant excretory rate prevails. Several patients who were clinically in excellent condition following implantation of pellets had corticosteroid levels which were well below the normal range and which were actually in the range found when these patients showed a marked insufficiency. It is probable that the constant and relatively slow rate of absorption of pellets leads to a much more efficient utilization and inactivation of the steroid than when the material is injected, with the consequent variable levels of circulating steroid. It is also likely that the lability of the pituitary adrenal cortex steroid secreting mechanism or of the end organ system for utilization of the hormones may be depressed by the presence of an unvarying amount of exogenous cortical steroids.

The ability of the patient with adrenal insufficiency to convert moderate amounts of DCA to a water-soluble steroid is most probably one of the means by which the body inactivates this substance. The recent demonstration by Hechter *et al.* (2) and Hayano and co-workers (3) that DCA can be converted by adrenal tissue to a substance having glycogen-depositing activity furnishes another example of conversion of DCA to a different steroid.

SUMMARY

1. Eleven patients with adrenal insufficiency have been studied by a method permitting measurement of "total corticosteroid," of "water-soluble corticosteroid," and of "corticosteroid poorly soluble in water."

2. The corticosteroid excretion of these subjects was low during the time when severe insufficiency was present, but during the interval without treatment before insufficiency developed, and during treatment, some of these patients excreted considerable amounts of corticosteroid. Administration of excessive amounts of DCA or cortical extract resulted in excretion of large amounts of corticosteroid.

3. When patients with adrenal insufficiency received DCA in moderate doses with consequent good clinical control, the corticosteroid excretion usually increased towards or reached the lower range of normal. This corticosteroid was water soluble. One patient who received an excessive amount of DCA excreted large amounts of corticosteroid which was almost entirely composed of the "corticosteroid poorly soluble in water." A small fraction of the injected DCA was recovered as corticosteroid in the urine when physiologic doses were administered, but when an excessive amount of DCA was given, a larger amount of the injected DCA was recovered.

REFERENCES

1. Lloyd, C. W., and Lobotsky, J.: Studies of urinary corticosteroid by a method permitting analysis of steroids poorly soluble in water. I. Normal adrenal function, *J. Clin. Endocrinol.* **10:** 1559–1569 (Dec.) 1950.
2. Hechter, O.; Jacobsen, R. P.; Jeanloz, R.; Levy, H.; Marshall, C. W.; Pincus, G., and Schenker, V.: The bio-oxygenation of 11-desoxycorticosterone at C-11, *J. Am. Chem. Soc.* **71:** 3261–3262 (Sept.) 1949.
3. Hayano, M.; Dorfman, R. I., and Prins, D. A.: Metabolism of the steroid hormones. Conversion of desoxycorticosterone to glycogenic material *in vitro*, *Proc. Soc. Exper. Biol. & Med.* **72:** 700–701 (Dec.) 1949.

THE EFFECT OF ADRENOCORTICOTROPIC HORMONE AND CORTISONE ON THE SERUM HEXOSAMINE LEVEL IN ACUTE DISSEMINATED LUPUS ERYTHEMATOSUS*

NORMAN F. BOAS, M.D.† AND LOUIS J. SOFFER, M.D.

From the Endocrine Section of the Medical Services and the Department of Chemistry, The Mount Sinai Hospital, New York

ACUTE disseminated lupus erythematosus is a systemic disease characterized by profound alterations of the connective tissue of the body (1). One of the striking changes is the presence of excess amounts of metachromatically-staining amorphous ground substance, which swells the tissues and separates the connective tissue fibers (2). Klemperer has identified this material histochemically as being composed largely of hyaluronic acid and probably chondroitin sulfuric acid (3).

Since hexosamine constitutes approximately 40 per cent of each of these mucopolysaccharides and since serum contains appreciable amounts of bound hexosamine (glucosamine and galactosamine),[1] it was deemed of interest to study the levels of circulating hexosamine in acute disseminated lupus erythematosus.

The Medical Services of the Mount Sinai Hospital have had the unusual opportunity of studying 20 patients with acute disseminated lupus erythematosus during the past year, 14 of whom were treated with either cortisone or adrenocorticotropic hormone (ACTH). Comprehensive reports of chemical and clinical studies of these patients appear elsewhere (4–6).

We have performed hexosamine determinations in 19 cases of lupus and have studied serially 11 patients who underwent treatment with cortisone and ACTH for periods as long as five months.

METHODS

Because of the relatively small supplies available, cortisone and ACTH were used only in patients who were critically ill and in whom an un-

Received for publication June 16, 1950.

* This study was aided by grants from the United States Public Health Service and Ciba Pharmaceutical Products, Inc.

† Charles Klingenstein Fellow, Mount Sinai Hospital. Present address: National Institute for Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.

[1] Hyaluronic acid contains glucosamine, and chondroitin sulfuric acid contains galactosamine. The determination for hexosamine in serum does not distinguish between these substances.

equivocal diagnosis of acute disseminated lupus erythematosus could be made.

Fasting morning blood samples were drawn, centrifuged and the serum kept refrigerated. West's modification for serum (7) of the method of Elson and Morgan (8) for the determination of hexosamine was used. All the dilutions and reactions, however, were carried out in 10 ml. glass-stoppered volumetric flasks. In our experience it was essential to redistill and recrystallize all reagents used. Each determination was run in duplicate. When differences were greater than 5 per cent the determinations were repeated. The average differences were between 0 and 3 per cent. Serum blanks (without acetylacetone) were not run routinely as recommended by Shetlar (9), since in our experience interfering colors did not develop. The final color was read in a single colorimeter tube at a wave length of 520 millimicrons. The serum hexosamine is expressed as milligrams per cent.

Our normal reference values were similar to those of West (7) and Rosenberg (10), and were based on determinations of samples of blood taken from 19 normal individuals. Our normal serum hexosamine levels varied from 85 to 138 mg. per cent with an average of 107 ± 13.4 mg. per cent.

RESULTS

In 10 of the 11 patients (Cases 1–10) studied serially, the serum hexosamine levels varied according to a definite pattern under the influence of ACTH and cortisone. Case 11 will be discussed separately. The initial serum hexosamine level, abnormally high in all these cases, invariably fell during treatment with both ACTH and cortisone. When the dosages of these hormones were reduced to a clinically ineffective level, the serum hexosamine level invariably increased, to fall again when the dosages were increased. In all the patients dosages were varied from week to week. In the 10 cases followed for many weeks there were 18 instances in which a clinically effective dose of either cortisone or ACTH caused a fall in the elevated serum hexosamine level and 19 instances in which, by decreasing the dose, an increase in the serum level occurred. Thus the level of circulating hexosamine could be altered at will by modifying the treatment dosage schedule (Fig. 1).

In the 4 cases studied at frequent intervals, the initial fall in the hexosamine level with ACTH developed after three, four and five days, respectively (Cases 7, 10 and 8) and with cortisone after eight days (Case 6). In the other instances, in which less frequent determinations were carried out, it can only be stated that the reduction took place between certain time intervals. In Cases 1–5 the fall took place in less than six, twenty, six, five, and nine days respectively, under the influence of cortisone and

in Case 9 in less than ten days with ACTH. Thus cortisone and ACTH seem to behave in the same way, effecting an initial fall in the serum hexosamine level somewhere between three days and a week from the beginning of treatment.

In 6 of the cases (Cases 6–11) studied at more frequent intervals, an additional phenomenon was observed which was probably missed in the other cases because less frequent determinations were made. In each there

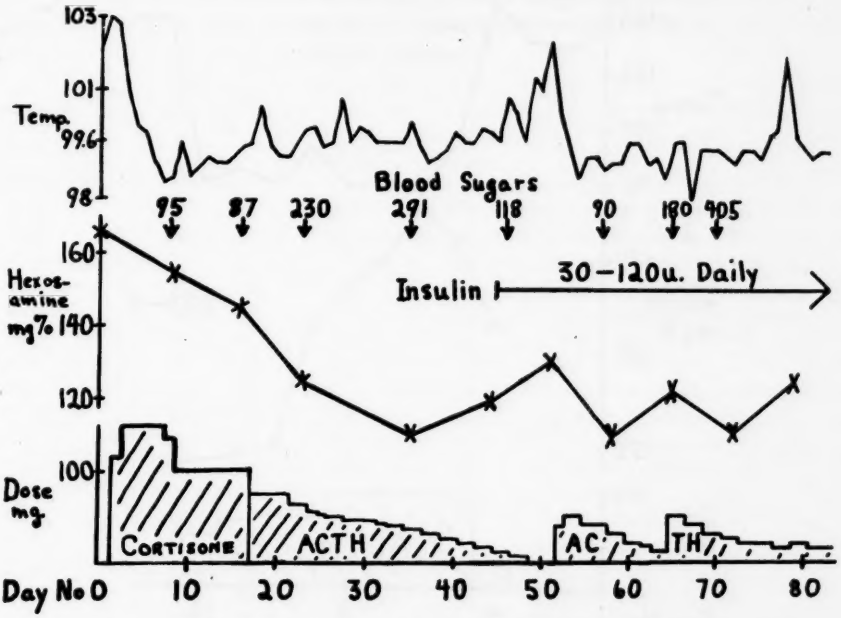

FIG. 1. Case 3. Showing alteration of the level of circulating hexosamine by modifying the dosage of cortisone and ACTH.

was an initial rise in the serum hexosamine level starting from one to three days after the beginning of therapy. The rise could easily be overlooked since it lasted only from one to five days and then was followed by the usual fall (Fig. 2).

In general it was evident that less ACTH (in milligrams) was necessary than cortisone to effect an equal lowering of the serum hexosamine level. The relative effectiveness of these compounds on the serum hexosamine level is paralleled by the observed clinical effects.

The hexosamine levels during the periods of most effective therapy fell within the normal range in only 1 instance (Case 3). In no case, however, was the disease actually cured.

Case 11 was not included in the above discussion since the hexosamine changes were at some variance with the above described pattern. This patient was extremely ill, but responded well clinically and in the usual way to ACTH and cortisone. The initial serum hexosamine level in this instance was within the normal range but following treatment a similar pattern was observed, although the changes in the hexosamine levels appeared to be somewhat delayed. It is of interest that hexosamine values

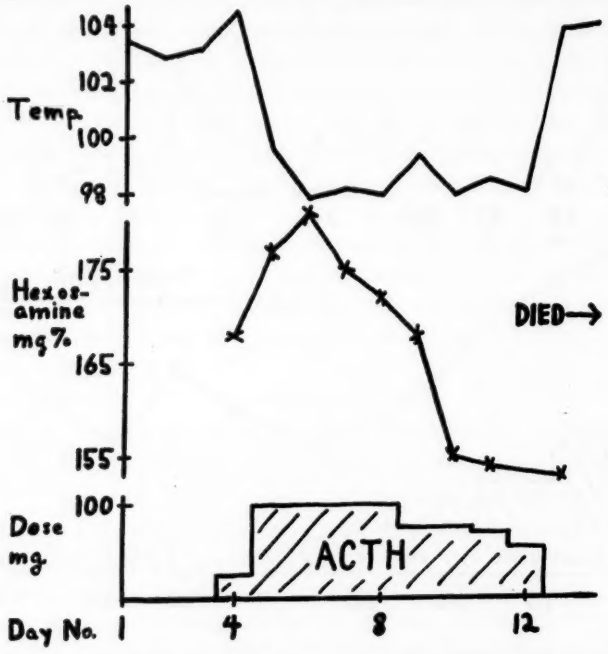

FIG. 2. Case 7. Showing transient rise in serum hexosamine level before the usual fall produced by ACTH therapy.

became elevated here only after reduction of the first therapeutically effective dose of ACTH.

The serum hexosamine levels and their response following treatment in the 3 males did not differ from those observed in the female patients.

In addition to the 11 patients studied serially, determinations were made of the serum hexosamine levels in 8 additional patients with acute disseminated lupus erythematosus. These patients did not receive hormone therapy (Table 1). All but one (L.R.) had high hexosamine values. L.R. was a boy who had a complicating nephrosis. He had a low total protein in the serum (4.61 Gm. per cent) with abnormally low albumin and gamma-

globulin fractions. His serum hexosamine level was within the normal range.

Blood glucose determinations were made in all the patients at weekly intervals. In all but 1 (Case 3) there were no changes. In Case 3 severe diabetes mellitus developed during therapy with ACTH, and blood glucose levels rose to as high as 405 mg. per cent. These changes in no way were related to the serum hexosamine levels.

TABLE 1

Patient	Case No.	Sex	Age (yrs.)	Serum hexosamine (mg. %)		Condition at end of study
				Before therapy	During therapy	
B.P.	1	F	32	234	156	Remission after therapy.
R.B.	2	M	31	184	158	Needs maintenance therapy.
W.E.	3	M	51	166	109	Needs maintenance therapy.
M.F.	4	F	36	234	122	Needs maintenance therapy.
F.W.	5	M	13	190	132	Needs maintenance therapy.
I.S.	6	F	22	160	124	Died during therapy.
J.D.	7	F	13	168	149	Died during therapy.
R.V.	8	F	20	181	146	Needs maintenance therapy.
N.M.	9	F	24	166	142	Needs maintenance therapy.
C.B.	10	F	34	166	148	Needs maintenance therapy.
S.L.	11	F	17	105	112	Needs maintenance therapy.
M.R.		F	25	220		Died without therapy.
R.B.		F	46	232		Died without therapy.
M.W.		F	35	235		Sick without therapy.
C.C.		F	23	208		Sick without therapy.
E.S.		F	32	215		Spontaneous remission later.
T.M.		F	29	204		Died without therapy.
M.S.		F	13	204		Died without therapy.
L.R.		M	17	123		Died without therapy.

DISCUSSION

Elevation of the serum hexosamine levels is not peculiar to disseminated lupus erythematosus, since it has been described in many conditions. It is elevated in many chronic disorders such as carcinomatosis, Hodgkin's disease, leukemia, rheumatoid arthritis, periarteritis nodosa, rheumatic fever and chronic infections (7, 11). It also rises in acute illnesses such as pneumonia (7, 11), acute myocardial infarction (7, 12), experimental infections and trauma (13). Rise of serum hexosamine may be produced by subcutaneous injection in rabbits of trypsin, chymotrypsin, lysozyme,

bovine serum albumin, casein (14), streptokinase (15), and hyaluronidase (16). Rosenberg and Schloss (10) have recently studied a series of cases of rheumatic fever and observed that levels which were high during the acute phase returned to normal with quiescence of the disease. It thus appears to be a temporary response called forth by tissue injury and may persist as long as the injury is present. The response has been variously interpreted as indicating tissue destruction or tissue proliferation (7, 13).

Since hexosamine is a normal component of the serum mucoproteins (17), its determination is in a sense an estimation of the total mucoproteins present. How this relationship is borne out in diseases in which abnormal changes take place in the mucoproteins is at present obscure.

The serum mucoproteins occur mostly in association with the serum globulins. Sørenson (18) has shown that the hexosamine concentration in globulin is four times that in albumin. The serum protein electrophoretic pattern in acute disseminated lupus erythematosus almost invariably shows an abnormal elevation of the alpha-2 and gamma-globulin fractions, and a decrease in the albumin fraction (6). The serum hexosamine changes may very likely be associated with one of these elevated fractions.[2]

Ragan (19) has recently pointed out that wounds in rabbits under the influence of ACTH and cortisone do not heal properly. One observed defect is the inability of the treated animal to deposit ground substance and mucopolysaccharides. This may in part explain the systemic improvement in lupus produced by these hormones, since there may occur a diminution in the excess ground substance present. Since serum hexosamine levels fall with ACTH and cortisone, there may be more than a casual relationship between the tissue and serum changes.

At present we cannot interpret our results, since relatively little is known of the source, metabolism and fate of hexosamine or mucoproteins. They are probably related to the depots of mucopolysaccharides throughout the body (such as cartilage, ground substance, mucous glands, vitreous humor, skin, and blood vessels).

It is evident from these observations, however, that this system, as reflected by the serum hexosamine, is profoundly influenced by hormones of the adrenal cortex.

SUMMARY

Seventeen out of 19 patients with acute disseminated lupus erythematosus were found to have elevated serum hexosamine values.

Eleven who were under treatment with ACTH and cortisone were studied serially for many weeks. In all, these hormones consistently low-

[2] We have recently demonstrated a highly significant positive correlation between the changes in serum hexosamine and the gamma-globulin fraction (N. F. Boas and M. Reiner: Effect of ACTH and cortisone on serum hexosamine and gamma-globulin changes in acute disseminated lupus erythematosis. To be published).

ered the serum hexosamine values during periods of clinically effective treatment.

REFERENCES

1. Klemperer, P.; Pollack, A. D., and Baehr, G.: Pathology of disseminated lupus erythematosus, *Arch. Path.* **32:** 569, 1941.
2. Klemperer, P.: The pathogenesis of lupus erythematosus and allied conditions, *Ann. Int. Med.* **28:** 1, 1948.
3. Klemperer, P.: Unpublished data.
4. Baehr, G., and Soffer, L. J.: Treatment of disseminated lupus erythematosus with cortisone and adrenocorticotropin, *Bull. N. Y. Acad. Med.* **26:** 229, 1950.
5. Soffer, L. J.; Boas, N. F.; Levitt, M.; Gabrilove, J. L., and Baehr, G.: The influence of cortisone and adrenocorticotropic hormone on acute disseminated lupus erythematosus, *Tr. A. Am. Physicians.* (In press.)
6. Reiner, M.: The effect of cortisone and adrenocorticotropic hormone on serum proteins in disseminated lupus erythematosus, *Proc. Soc. Exper. Biol. & Med.* **74:** 529, 1950.
7. West, R., and Clarke, D. H. The concentration of glucosamine in normal and pathological sera, *J. Clin. Investigation* **17:** 173, 1938.
8. Elson, A. L., and Morgan, W. T. J.: A colorimetric method for the determination of glucosamine and chondrosamine, *Biochem. J.* **27:** 1824, 1933.
9. Shetlar, M. R.; Foster, J. V.; Kelly, K. H., and Everett, M. R.: Serum polysaccharide level in the normal state, *Proc. Soc. Exper. Biol. & Med.* **69:** 507, 1948.
10. Rosenberg, C., and Schloss, B.: Plasma hexosamine levels in acute rheumatic fever, *Am. Heart J.* **38:** 872, 1949.
11. Winzler, R. J., and Smith, I. M.: Studies on the mucoproteins of human plasma. II. Plasma mucoprotein levels in cancer patients, *J. Clin. Investigation* **27:** 617, 1948.
12. Simkin, B.; Ergman, H. C., and Prinzmetal, M.: Studies on coronary circulation V. Quantitative changes in a serum mucoprotein following the occurrence of myocardial infarction, *Am. J. Med.* **6:** 734, 1949.
13. Shetlar, M. R.; Bryan, R. S.; Foster, J. V.; Shetlar, C. L., and Everett, M. R.: Serum polysaccharide levels in experimental inflammation, *Proc. Soc. Exper. Biol. & Med.* **72:** 294. 1949.
14. Schlamowitz, S. T.; DeGraff, A. C., and Schubert, M.: Studies of tissue response to injections of enzymes. II. Changes in rabbit plasma and tissue hexosamine induced by a single subcutaneous injection of trypsin, *Circulation* **1:** 822, 1950.
15. Schlamowitz, S. T.; DeGraff, A. C., and Schubert, M.: Studies of tissue response to injections of enzymes. III. Preliminary report. Development of subcutaneous "nodules" and tissue hexosamine changes in rabbits following a single subcutaneous injection of streptokinase, *Circulation* **1:** 833, 1950.
16. Schlamowitz, S. T.; Morrison, E., and Schubert, M.: Studies of tissue response to injections of enzymes. IV. Tissue, hexosamine, and hematologic changes in rabbits following a single subcutaneous injection of hyaluronidase, *Circulation* **1:** 838, 1950.
17. Winzler, R. J.; Devor, A. W.; Mehl, J. W., and Smith, I. M.: Studies on the mucoproteins of human plasma. I. Determination and isolation, *J. Clin. Investigation* **27:** 609, 1948.
18. Sørenson, M.: Determination of glucosamine in protein, *Compt. rend. de trav. du lab. Carlsberg, série chim.* **22:** 487, 1938.
19. Ragan, C.; Howes, E. L.; Plotz, C. M.; Meyer, K.; Blunt, J. W., and Lattes, R.: The effect of ACTH and cortisone on connective tissue, *Bull. N. Y. Acad. Med.* **26:** 251, 1950.

THE EFFECT OF ADRENALIN ON ADRENAL CORTICAL FUNCTION*

EUGENE L. BLISS, M.D.,† SIDNEY RUBIN, M.D. AND THEODORE GILBERT, M.A.,
with the technical assistance of
MRS. RUTH MILLER

From the Winter Veterans Administration Hospital, Topeka, Kansas

RECENT evidence indicates that adrenalin will activate the adrenal cortex in animals and man. Vogt (1) demonstrated that adrenalin produces a significant increase in the corticoids in eviscerated dogs and cats, and Long (2, 3) showed that large amounts activate the adrenal cortex in rats. Sayers and Sayers (4) confirmed this in rats. Recently, Recant *et al.* (5) and Thorn *et al.* (6), administered adrenalin to humans and demonstrated increased adrenal cortical function.

It was the purpose of this investigation to determine the importance of adrenalin as an adrenal cortical activator, by studying its influence on those hematologic elements, urinary products and blood chemicals which are affected by the hormones of the adrenal cortex.

METHODS

All subjects, unless otherwise specified, were normal. They comprised aides, doctors, nurses and selected patients. The patients who were chosen were in the hospital either for nonpsychotic emotional difficulties in which the element of anxiety was minimal; for minor medical problems such as old injuries, or mild aches and pains; or awaiting discharge after recovering completely from some acute febrile illness.

No attempt was made to control diet or activity, save in the case of one patient who was studied on a metabolic regimen. However, with short-term experiments, patients were studied in the postprandial state, the last food having been consumed at 6:00 P.M. the preceding evening.

Chemical studies were made on 16 subjects over two-day and three-day periods. Twenty-four-hour urines were collected. The first day's sample served as the control. On the second day, 1 cc. of adrenalin in oil (2 milli-

Received for publication October 28, 1949.

* Sponsored by the Veterans' Administration and published with the approval of the chief medical director. The statements and conclusions published by the authors are results of their own study and do not necessarily reflect the opinion or policy of the Veterans' Administration.

† Present address: Department of Psychiatry, College of Medicine, University of Utah, Salt Lake City, Utah.

grams) was administered intramuscularly at 8:00 A.M., 12:00 noon, 4:00 P.M. and 8:00 P.M.—a total of 8 milligrams over a period of twelve hours. This particular time interval and dosage were selected arbitrarily. A 24-hour collection was made the third day in 12 cases. All urine specimens were collected at appropriate intervals and stored in the refrigerator. When 17-ketosteroid determinations were to be made, the urine was acidified with concentrated HCl immediately and then placed in the refrigerator. Specimens intended for uric acid analyses were frozen whenever immediate determinations were impractical.

Hematologic studies were made on venous blood, drawn with minimal stasis and preserved in balanced oxalate. White blood count and differential counts were done by routine methods. Eosinophil counts were performed by the modification of the direct method of Dunger, suggested by Forsham *et al.* (7). The following chemical methods were used:

17-Ketosteroids were determined by the method of Cahen and Salter (8); uric acid by Archibald's modification of Kern and Stransky's method, cited by Forsham *et al.* (7); creatinine by Folin's method (9); whole blood ascorbic acid by the method of Roe and Kuether (10), using bromine instead of norite as oxidant; total cholesterol by the method of Sperry and Brand (11); urinary potassium by the method of Blanchetiere and Pirlot as modified by Albanese and Wagner (12); and urinary sodium by the colorimetric triple acetate method described by Albanese and Lein (13). Optical densities were measured with the Coleman Junior spectrophotometer, Model 6, for all methods except that for cholesterol; the Klett-Summerson instrument was used in the latter determination; urinary chlorides were determined by the Volhard method.

RESULTS

Twenty-four-hour urine studies

1. *Changes in excretion of 17-ketosteroids:* There was a mean rise in 17-ketosteroid excretion of 46 per cent on the second day (the day adrenalin was administered) in the 12 normal subjects examined.

A wide fluctuation in the 17-ketosteroid excretion occurred in several instances on the day following the injection of adrenalin (third day), suggesting a disturbance in basic glandular rhythm. There were both increases and decreases, resulting in a mean change of 0 per cent when compared to the values on the first day. Four subjects were studied on a regimen of 1 mg. of adrenalin in oil three times a day, but the changes in 17-ketosteroid excretion were not significant (Table 1).

Although 24-hour 17-ketosteroid excretion is not constant from day to day (14, 15), Talbot (16) notes that the chances are two to one that any single assay will fall within 15 per cent of the 17-ketosteroid value obtained

by averaging 30 or 40 consecutive daily assays. A 46 per cent mean rise, therefore, would seem to be significant. Complete figures for 17-ketosteroid, as well as the uric acid and creatinine eliminations, are included in Table 7.

There seemed to be no clear or simple correlation between the degree of disturbance produced by adrenalin in the way of palpitations, tremor, headache, or apprehension, and the degree to which the 17-ketosteroid rise occurred. As an example, "BR" experienced only slight, transient palpitations and yet his ketosteroid excretion rose from 18.5 mg. on the control day to 29 mg. on the test day. "WO," however, suffered from severe palpitations, a constricting headache and disquieting nervousness during the entire test day, yet there was no change in ketosteroid titer.

One patient with Addison's disease was subjected to the same procedure and failed to show a significant change.

TABLE 1. CHANGES IN THE DAILY EXCRETION OF 17-KETOSTEROIDS WITH ADRENALIN

GROUPS	NUMBER SUBJECTS	MEAN VALUES AND RANGE			SUBJECTS SHOWING INCREASE	% CHANGE AND RANGE		
		CONTROL DAY	TEST DAY ADRENALIN ADMINISTERED	DAY AFTER TEST		CONTROL DAY	TEST DAY ADRENALIN ADMINISTERED	DAY AFTER TEST
NORMAL 1 MG. ADRENALIN IN OIL TID	4	15.5 4.8 TO 23.4	15.8 5.5 TO 22.7		2	0%	5% -5% TO +15%	
NORMAL 2 MG. ADRENALIN IN OIL QID	12	15.5 5.1 TO 27.4	19.9 9.3 TO 32.8	14.0 6.7 TO 24.8	11	0%	46% -19% TO +188%	0% -49% TO 90%
ADDISONIAN 2 MG. ADRENALIN IN OIL QID	1	4.7	5.3		1	0%	13%	

← MG PER 24 HOURS →

Since the 17-ketosteroids in the male, are said to derive from the testes as well as the adrenal cortex, the rise in titer might well have been due to a stimulation of the testes rather than the adrenal cortex. Therefore, 3 females were included in the series in an effort to eliminate this possibility. The female subjects received four-fifths of the adrenalin dose (1.6 mg. in oil) for normal males. Two of the 3 female subjects showed a considerable increase on the test day, suggesting that adrenalin does stimulate the adrenal cortex, since this is the principal source of these steroids in the female. Moreover, these rises were of the same order as those observed in the males, suggesting that any stimulation of the testes which might have occurred in the males was minimal. In addition, the failure of the Addisonian patient to achieve a significant increase would also argue against a stimulation of the testes by adrenalin.

2. *Uric acid excretion:* It has been reported (17, 18, 19) that adrenal cortical activity results in an increased uric acid elimination in the urine. The 12 normal subjects studied demonstrated a mean rise in uric acid on the test day, of 48 per cent (Table 2).

TABLE 2. CHANGES IN URINARY URIC ACID OVER 24-HOUR PERIODS WITH 2 MG. ADRENALIN IN OIL Q.I.D.—GRAMS/24 HOURS

GROUPS	NUMBER SUBJECTS	MEAN VALUES IN GRAMS AND RANGE			SUBJECTS SHOWING INCREASE	% CHANGE AND RANGE		
		CONTROL DAY	TEST DAY ADRENALIN ADMINISTERED	DAY AFTER TEST		CONTROL DAY	TEST DAY ADRENALIN ADMINISTERED	DAY AFTER TEST
NORMAL	12	.71 .36 TO 1.30	1.00 .66 TO 1.60	.71 .31 TO 1.20	12	0%	+48% 166% TO +6%	+4% +91% TO -52%
ADDISONIAN	1	.67	.66		0	0%	-1%	

Since no increase in uric acid excretion occurred with adrenalin in the case of Addison's disease, it seemed possible that the increase found in the normal subjects derived from adrenal cortical activity.

Short-term studies over four-hour periods were made to determine whether the immediate rise in urinary uric acid that occurs with ACTH (7) is duplicated by adrenalin. Unlike ACTH, adrenalin (2 mg. in oil) did not produce an immediate rise; if anything, there was a slight, but not statistically significant, drop of 10 per cent (Table 3). At the same time, the creatinine excretion remained constant.

TABLE 3. CHANGES IN URINARY URIC ACID OVER A 4-HOUR PERIOD WITH 2 MG. ADRENALIN IN OIL—GRAMS/4 HOURS

GROUP	NUMBER SUBJECTS	MEAN VALUE & RANGE		NO. SUBJECTS SHOWING INCREASE	% CHANGE & RANGE
		CONTROL 4-HOUR PERIOD	AFTER ADRENALIN 4-HOUR PERIOD		
NORMAL	9	.136 .027 TO .301	.109 .025 TO .143	3	-10% +40% TO -39%

3. *Creatinine excretion:* There was an appreciable increase in twenty-four-hour creatinine elimination on the adrenalin day in every instance (Table 8). This contrasts with the slight drop or constant excretion usually found with ACTH (20, 21).

4. Two subjects, "WI" and "DU," gave chemical responses which were unusual. In both instances, creatinine and uric acid excretions on the test day were far below those of the control day. At first it was thought that this might be due to faulty urinary collections, but re-investigation failed to uncover any error. Chemical assays also were repeated and verified. Even in these cases, however, there was a relative increase in 17-keto-steroids as compared with uric acid and creatinine values (Table 7).

Four-hour blood studies

Changes in the chemistry and hematologic values of the blood were studied in fasting subjects over a four-hour period, from 8:00 A.M. to 12:00 noon.

1. *Serum cholesterol and whole blood vitamin C:* Changes in the concentration of cholesterol and vitamin C in the adrenals of animals have been used as a measure of adrenal cortical activity (22, 23, 24).

We performed four-hour studies in order to determine whether significant blood changes might occur over this brief period, with adrenalin. Normal subjects demonstrated a slight drop in blood vitamin C following the administration of 2 mg. of adrenalin in oil. Two separate tests were run on the single case of Addison's disease and both revealed a drop identical with that of the normal subjects (Table 4).

TABLE 4. CHANGES IN WHOLE BLOOD ASCORBIC ACID OVER A 4-HOUR PERIOD

GROUPS	NUMBER SUBJECTS	MEAN OF BLOOD ASCORBIC ACID & RANGES IN MG % 8 AM CONTROL	4 HRS AFTER ADRENALIN 12 NOON	PERCENTAGE DIFFERENCE
NORMAL CONTROL	8	1.16 .56 TO 1.70	1.17 .54 TO 1.76	+1%
NORMAL 2 MG. ADRENALIN IN OIL	7	1.16 .52 TO 2.23	1.08 .42 TO 1.85	-7%
ADDISONIAN 2 MG. ADRENALIN IN OIL	1 2 TESTS	1.19 1.08 & 1.31	1.09 1.02 & 1.17	-8%

Similar studies with serum cholesterol failed to show any significant changes in either the normal subject or the patient with Addison's disease over this brief time span (Table 5).

TABLE 5. CHANGES IN SERUM CHOLESTEROL OVER A 4-HOUR PERIOD

GROUPS	NUMBER SUBJECTS	MEAN OF SERUM CHOLESTEROL & RANGE IN MG % 8 AM CONTROL	4 HRS AFTER ADRENALIN 12 NOON	PERCENTAGE DIFFERENCE
NORMAL CONTROL	7	271 210 TO 330	269 207 TO 336	-1%
NORMAL 2 MG. ADRENALIN IN OIL	10	276 235 TO 346	272 230 TO 345	-2%
ADDISONIAN 2 MG. ADRENALIN IN OIL	1	162	166	+2%

2. *Eosinophils:* Variations in the total number of blood eosinophils would appear to be an excellent guide to adrenal cortical function (7).

CHANGE IN TOTAL EOSINOPHILS OVER A 4 HOUR PERIOD AFTER 2 MG. OF ADRENALIN IN OIL

GROUP	NUMBER SUBJECTS	MEAN TOTAL EOSINOPHILS PER CU. MM AND RANGE 8 AM	12 NOON	PERCENTAGE CHANGE	RANGE
NORMAL	15	250 63 TO 441	61 0 TO 213	-79%	-100 % to -52%
ORGANIC	19	244 81 TO 600	119 6 TO 300	-60%	-93% to -33%
SCHIZOPHRENIC	28	175 62 TO 306	50 0 TO 150	-71 %	-100% to -41 %
ADDISONIAN	1 (3 TESTS)	200 119 TO 250	162 100 TO 206	-17 %	-27 % to -6 %
		CONTROL	4 HRS. AFTER ADRENALIN		

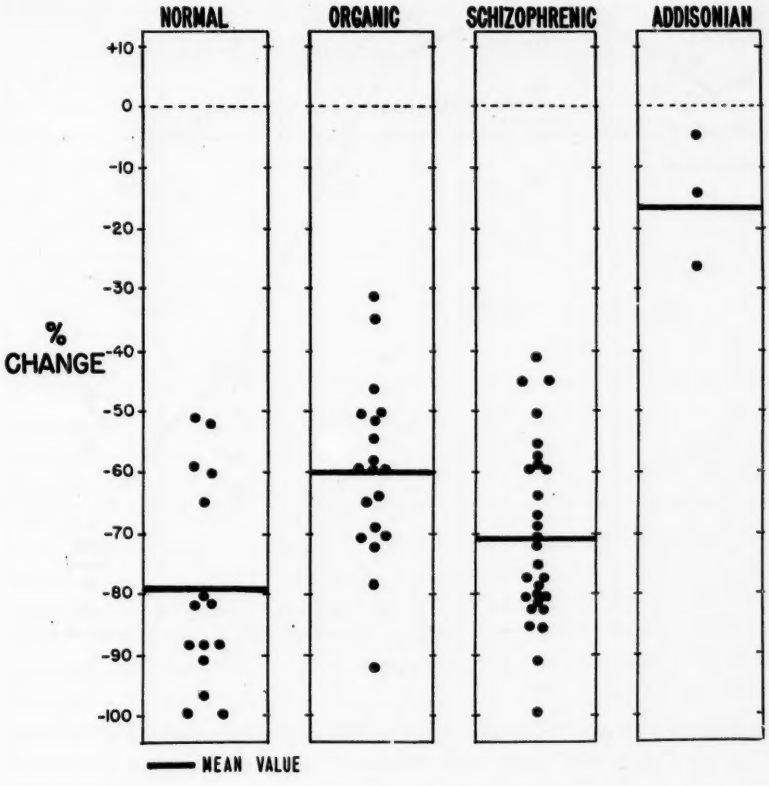

FIGURE 1

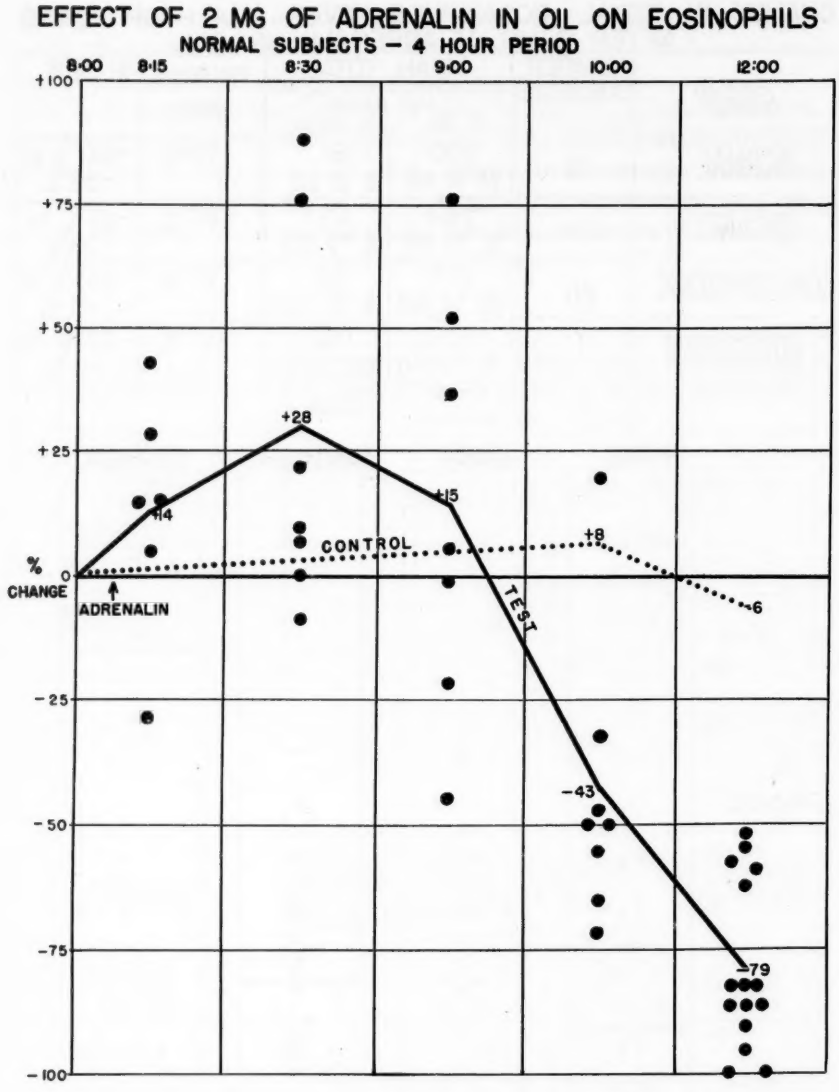

FIGURE 2

Adrenalin will cause the same drop in eosinophils as does ACTH but, according to Thorn (25), not with the same regularity. Using 0.3 mg. of adrenalin in water subcutaneously, he reports that only 1 patient with Addison's disease has, in his experience, shown an appreciable drop. This

has been confirmed by Laragh *et al.* (26), who reported a similar experience with fewer cases.

Two milligrams in oil was selected as a standard dose and a series of normal subjects, a series of chronically and acutely ill patients ("organic"), 1 Addisonian, and a group of schizophrenics were investigated and their eosinophilic responses determined. Normal subjects and schizophrenics showed the same scatter and degree of diminution in counts. A few of the chronically ill patients showed only moderate decreases, but the majority showed decreases in the normal range. Only the Addisonian showed a negligible reduction (Fig. 1). This would appear to confirm the observations of the other investigators.

TABLE 6. CHANGES IN CIRCULATING LYMPHOCYTES OVER A 4-HOUR PERIOD WITH ADRENALIN

	GROUP	NUMBER SUBJECTS	MEAN TOTAL LYMPHOCYTES		PERCENTAGE CHANGE
			CONTROL 8 A.M.	12 NOON	
	NORMAL CONTROL	7	3261	4103	+26%
GROUPS RECEIVING ADRENALIN	NORMAL .7 MG ADRENALIN IN H_2O	8	3282	2922	-14%
	NORMAL 2 MG ADRENALIN IN OIL	8	3630	4143	+15%
	NORMAL 2 MG. ADRENALIN IN OIL PRECEDED 1/2 HOUR BY 50 MG PRISCOLINE IM	6	3627	3640	+1%

4 HRS. AFTER ADRENALIN

The rate of decline of the total eosinophils over a four-hour period was established by taking blood samples at one-quarter hour, one-half hour, one hour, two hours, and four hours, after the injection of 2 mg. of adrenalin in oil. In Figure 2 the curve has been plotted.

3. *Lymphocytes:* A diminution in the total blood lymphocytes has been used as an indicator of adrenal cortical activity (20). Further experience with ACTH has confirmed these earlier observations (7), but has indicated that the change in the total lymphocyte count is a less reliable guide to adrenal cortical activity than the change in total eosinophils. Recently, Parson *et al.* (27) found a slight drop in total lymphocyte count over a four-hour period when adrenalin was administered. Gabrilove *et al.* (28) described a similar decrease in normal subjects, but found that an identical drop occurred in a group of Addisonians when the same amount of adrenalin (0.7 mg. in water i.m.) was administered.

TABLE 7. TWENTY-FOUR-HOUR URINE STUDIES

Patient and sex	Day*	Urine volume, cc.	Creatinine, Gm./24 hrs.	Uric acid, Gm./24 hrs.	17-ketosteroids, mg./24 hrs.
HE	1	900	1.5	.68	15.4
(M)	2	1090	1.7	.77	12.5
SU	1	1260	1.8	.71	15.9
(M)	2	2760	2.2	1.30	19.0
	3	2390	1.9	.74	18.1
BU	1	1370	2.1	1.34	18.6
(M)	2	2585	2.4	1.60	22.1
	3	1145	1.8	1.24	9.5
GI	1	1290	1.3	.41	14.8
(M)	2	1750	2.0	.70	22.6
	3	1410	1.8	.57	15.4
BE	1	1465	1.4	.60	5.1
(M)	2	3420	1.7	1.56	14.7
	3	1510	1.4	.73	9.7
BR	1	1610	2.0	.84	18.5
(M)	2	2830	2.1	.89	29.0
	3	2015	1.9	.71	11.0
GR	1	930	1.7	.92	27.4
(M)	2	1650	2.2	.99	32.8
	3	1480	1.8	.86	24.8
HEA	1	2970	2.0	.95	25.9
(M)	2	2740	2.1	1.10	27.0
	3	2210	2.0	.89	24.3
FI	1	1070	1.1	.63	6.3
(M)	2	1930	1.4	.72	9.3
	3	870	1.2	.60	6.7
CU	1	640	.8	.64	10.9
(F)	2	1460	.9	.73	17.5
	3	740	.4	.31	8.5
DU	1	1285	.8	.36	13.5
(F)	2	1600	1.2	.66	20.7
	3	900	.8	.34	13.7

TABLE 7—(*continued*)

Patient and sex	Day*	Urine volume, cc.	Creatinine, Gm./24 hrs.	Uric acid, Gm./24 hrs.	17-ketosteroids, mg./24 hrs.
WO (F)	1	835	.9	.43	11.1
	2	1370	1.2	.99	11.3
	3	980	1.0	.80	12.0
DO† (M)	1	670	1.3	.42	8.1
	2	1160	.9	.30	10.2
Addison's disease					
GA (M)	1	1265	1.5	.67	4.7
	2	1330	1.5	.66	5.3
Exceptions					
WI‡ (M)	1	1020	1.6	.74	13.1
	2	2080	.9	.42	12.3
DU‡ (M)	1	2080	1.5	.58	12.7
	2	2040	.5	.43	10.9
	3	2300	1.1	.50	13.6

* First day—Control day
Second day—1 cc. adrenalin in oil q.i.d.
Third day—Day after adrenalin
† Case excluded—incomplete urinary collection on second day.
‡ Case excluded—anomalous data—note creatinine and uric acid.

A variety of dosages of adrenalin in water, in oil, and combined with priscoline was used. All failed to produce a significant drop in the total circulating lymphocytes over a four-hour period. (Table 6).

Metabolic balance studies

One subject, B.T., who suffered from an active rheumatoid arthritis, was given a constant diet, which was standardized in respect to protein, calories, sodium and potassium. After a control period of approximately two weeks, during which urinary 24-hour nonprotein nitrogen and chloride excretion were followed until they became relatively stable, adrenalin (2 mg. in oil q.i.d.) was administered. Unfortunately, the effects of adrenalin were so disturbing that the patient requested it be discontinued after three days. A post-adrenalin control period could not be obtained. The significant findings were an initial increased elimination of sodium and

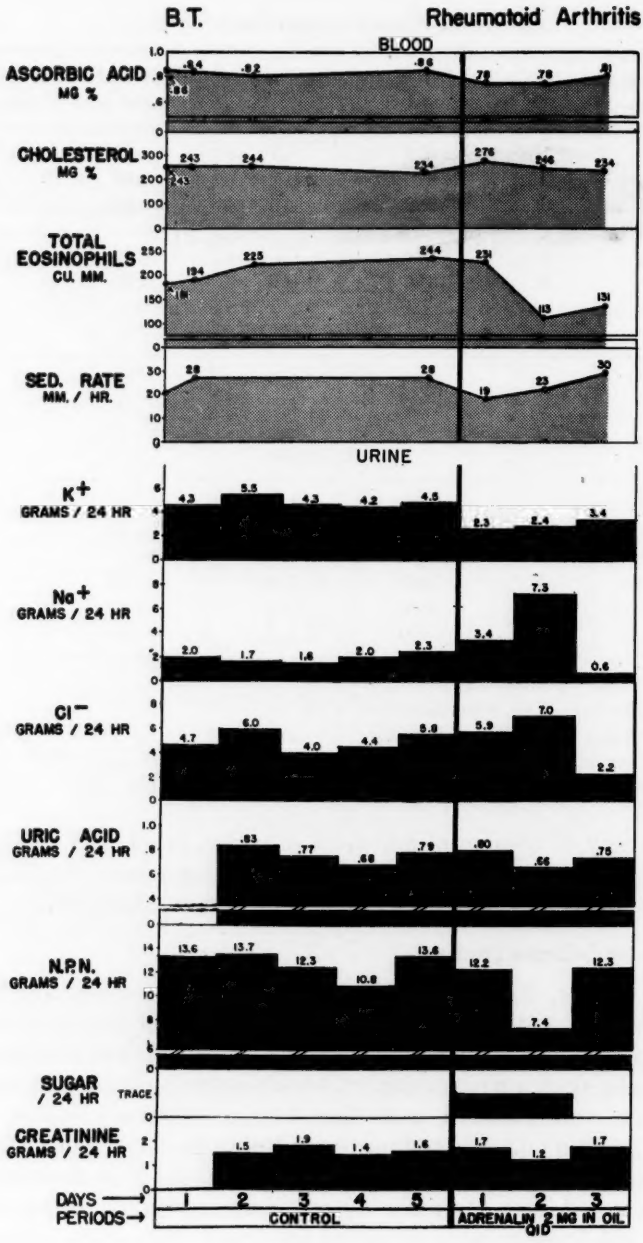
B.T.
Rheumatoid Arthritis
BLOOD
ASCORBIC ACID MG %
CHOLESTEROL MG %
TOTAL EOSINOPHILS CU. MM.
SED. RATE MM. / HR.
URINE
K+ GRAMS / 24 HR
Na+ GRAMS / 24 HR
Cl− GRAMS / 24 HR
URIC ACID GRAMS / 24 HR
N.P.N. GRAMS / 24 HR
SUGAR / 24 HR
TRACE
CREATININE GRAMS / 24 HR
DAYS →
PERIODS →
1 2 3 4 5
CONTROL
1 2 3
ADRENALIN 2 MG IN OIL
QID

FIGURE 3

chloride, and the retention of potassium. On the third day there was a retention of sodium and chloride in response probably to the previous losses. In addition, there was a moderate drop in total circulating eosinophils and a minimal drop in blood ascorbic acid. On two test days there was a trace of sugar in the urine. Changes in uric acid excretion as well as the sedimentation rate and serum cholesterol were not significant. The patient was not on this regimen long enough to determine the therapeutic value of adrenalin in rheumatoid arthritis (Fig. 3).

DISCUSSION

Since adrenal cortical activity bears an intimate relationship to so many disease processes, it has become increasingly important to understand more clearly how diverse forms of stress and strain are translated into stimuli to adrenal cortical function and what role adrenalin may play in this process.

Our data would indicate that the administration of adrenalin produces definite although slight stimulation of the adrenal cortex in man. Adrenalin causes a significant increase in urinary 17-ketosteroids but less than that usually obtained with ACTH. A large decrease in the total eosinophils over a four-hour period also occurs, a change which is negligible in patients with Addison's disease. In addition, a significant rise in daily uric acid excretion takes place, which is also negligible in Addisonian patients.

There are striking differences, however. The immediate rise in uric acid excretion over a four-hour period, which is so characteristic following administration of ACTH, does not occur with adrenalin. If anything, there is a slight diminution. As for the electrolytes, a reversal of the adrenal cortical effect was observed in the one subject studied on a metabolic regimen. There was an increase in sodium and chloride elimination and a diminution in potassium excretion, a pattern common to underactivity of the cortex rather than to overactivity. In addition, a significant glycosuria did not occur when adrenalin was given for three days. This contrasts with the marked glycosuria which occurs at times with ACTH (19).

There might be some question as to whether or not the dosages of adrenalin used were physiologic. In this connection, C. N. H. Long (29) stated that the quantities probably approximated the maximum blood levels that are achieved by direct discharge of adrenalin from the gland.

The data suggest that adrenalin is a partial activator of the adrenal cortex and will reproduce some, but not all, of the effects of ACTH. Although adrenalin can be a potent force in the mobilization of adrenal cortical hormones, it is probably only one of several such agents and is not the prime mover in the activation of this endocrine complex.

SUMMARY

1. Adrenalin has been administered intramuscularly to determine its effect upon adrenal cortical function in man.

A. Effect upon androgenic fraction:

1. There was a mean increase of 46 per cent in the daily excretion of 17-ketosteroids in 12 normal subjects when adrenalin (2 mg. in oil four times a day) was administered. Females as well as males were included in the series. A patient with Addison's disease failed to show a significant increase.

B. Effect upon "11 oxysteroid" fraction:

1. There was a mean increase of 48 per cent in the 24-hour urinary uric acid excretion in 12 normal subjects when adrenalin (2 mg. in oil four times a day) was administered. The Addisonian showed no increase.

2. There was no increase in urinary uric acid excretion over a four-hour period following adrenalin (2 mg. in oil), in contrast to the increase reported with ACTH.

3. There was a mean decrease in total circulating eosinophils over a four-hour period following adrenalin (2 mg. in oil), which amounted to 79 per cent in normal subjects, 71 per cent in a group of schizophrenic patients, and 60 per cent in a group of acutely and chronically ill medical patients. The Addisonian patient showed a negligible drop of 17 per cent.

4. Adrenalin failed to produce a significant lymphopenia over a four-hour period.

C. Effect upon electrolytic fraction:

A subject with an active rheumatoid arthritis was studied on a metabolic regimen. Adrenalin (2 mg. in oil four times a day) was administered for three days and resulted in an increased excretion of sodium and retention of potassium, a reversal of the normal "desoxy" action.

D. Miscellaneous effects:

Serum cholesterol did not vary over a four-hour period after adrenalin (2 mg. in oil) was given. Whole blood ascorbic acid dropped slightly after the same procedure. An identical change occurred in the Addisonian patient, suggesting that the decrease was not an adrenal cortical effect.

2. Adrenalin appears to be a potent but only partial activator of the adrenal cortex. It does not seem to be the agent principally responsible for the activation of the pituitary adrenal cortical complex.

Acknowledgments

We wish to thank William Campbell for the drawing of charts and graphs and Dr. George Cartwright and Dr. Nathaniel Uhr for their helpful criticisms.

REFERENCES

1. Vogt, M.: Observations on some conditions affecting the rate of hormone output by the suprarenal cortex, *J. Physiol.* **103:** 317–332 (Dec. 15) 1944.
2. Long, C. N. H.: Recent studies on the function of the adrenal cortex, *Bull. New York Acad. Med.* **23:** 260–282 (May) 1947.
3. Long, C. N. H., and Fry, E. G.: Effect of epinephrine on adrenal cholesterol and ascorbic acid, *Proc. Soc. Exper. Biol. & Med.* **59:** 67–68 (May) 1945.
4. Sayers, G., and Sayers, M. A.: Regulation of pituitary adrenocorticotrophic activity during the response of the rat to acute stress, *Endocrinology* **40:** 265–273 (Apr.) 1947.
5. Recant, L.; Forsham, P. H., and Thorn, G. W.: Observations on pituitary-adrenal response following epinephrine infusion in man, (abstract) *J. Clin. Endocrinol.* **8:** 589–590 (July), 1948.
6. Thorn, G. W.; Bayles, T. B.; Massell, B. F.; Forsham, P. H.; Hill, S. R.; Smith, S., and Warren, J. E.: Medical progress: studies on the relation of pituitary-adrenal function to rheumatic disease, *New England J. Med.* **241:** 529–537 (Oct. 6) 1949.
7. Forsham, P. H.; Thorn, G. W.; Prunty, F. T. G., and Hills, A. G.: Clinical studies with pituitary adrenocorticotropin, *J. Clin. Endocrinol.* **8:** 15–66 (Jan.) 1948.
8. Cahen, R. L., and Salter, W. T.: Urinary 17-ketosteroids in metabolism: standardized chemical estimation, *J. Biol. Chem.*, **152:** 489–499 (March) 1944.
9. Folin, W. M., and Wu, H.: A system of blood analysis, *J. Biol. Chem.* **38:** 81, 1919.
10. Roe, J. H., and Kuether, C. A.: Determination of ascorbic acid in whole blood and urine through 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid, *J. Biol. Chem.* **147:** 399–407 (Feb.) 1943.
11. Sperry, W. M., and Brand, F. C.: Colorimetric determination of cholesterol, *J. Biol. Chem.* **150:** 315–324 (Oct.) 1943.
12. Albanese, A. A., and Wagner, D. C.: A method for the colorimetric determination of potassium in biological products, *J. Lab. & Clin. Med.* **30:** 280–284 (March) 1945.
13. Albanese, A. A., and Lein, M.: The microcolorimetric determination of sodium in human biological fluids, *J. Lab. & Clin. Med.* **33:** 246–250 (Feb.) 1948.
14. Forbes, A. P.; Donaldson, E. C.; Reifenstein, E. C., Jr., and Albright, F.: The effect of trauma and disease on the urinary 17-ketosteroid excretion in man, *J. Clin. Endocrinol.* **7:** 264–288 (April) 1947.
15. Werner, S. C.: The daily variation in 17-ketosteroid excretion of men and women, *J. Clin. Endocrinol.* **1:** 951–954 (Dec.) 1941.
16. Talbot, N. B., and Butler, A. M.: Urinary 17-ketosteroid assays in clinical medicine, *J. Clin. Endocrinol.* **2:** 724–729 (Dec.) 1942.
17. Forsham, P. H.; Thorn, G. H.; Bergner, G. E., and Emerson, K.: Metabolic changes induced by synthetic 11-dehydrocorticosterone acetate, *Am. J. Med.* **1:** 105–134 (Aug.) 1946.
18. Thorn, G. W.; Prunty, F. T. G., and Forsham, P. H.: Changes in urinary steroid excretion and correlated metabolic effects during prolonged administration of adrenocorticotropic hormone in man, *Science* **105:** 528, (May 16) 1947.

19. Conn, J. W.; Lewis, L. H., and Wheeler, C. E.: Production of temporary diabetes in man with pituitary adrenocorticotropic hormone: relation to uric acid metabolism, *J. Lab. & Clin. Med.* **33:** 651–661 (June) 1948.
20. Dougherty, T. F., and White, A.: Influence of hormones on lymphoid tissue structure and function. The role of the pituitary adrenotrophic hormone in the regulation of the lymphocytes and other cellular elements of the blood, *Endocrinology* **35:** 1–14 (July) 1944.
21. Soffer, L. J.; Gabrilove, J. L.; Laqueur, H. P.; Volterra, M.; Jacobs, M. D., and Sussman, M. L.: The effects of anterior pituitary adrenocorticotropic hormone (ACTH) in myasthenia gravis with tumor of the thymus, *Jr. Mt. Sinai Hosp.* **15:** 73–82 (July–August) 1948.
22. Sayers, G.; Sayers, M. A.; Liang, T. Y., and Long, C. N. H.: The effect of pituitary adrenotrophic hormone on the cholesterol and ascorbic acid content of the adrenal of the rat and the guinea pig, *Endocrinology* **38:** 1–9 (Jan.) 1946.
23. Sayers, G.; Sayers, M. A.; White, A., and Long, C. N. H.: The effect of pituitary adrenotrophic hormone on cholesterol content of rat adrenal glands, *Proc. Soc. Exper. Biol. & Med.* **52:** 200–202 (March) 1943.
24. Mason, H. L.; Power, M. H.; Rynearson, E. H.; Ciaramelli, L. C.; Li, C. H., and Evans, H. M.: Results of administration of anterior pituitary adrenocorticotropic hormone to a normal human subject, *J. Clin. Endocrinol.* **8:** 1–14 (Jan.) 1948.
25. Thorn, G. W.: Personal communication.
26. Laragh, J. H., and Almy, T. P.: Changes in circulating eosinophils in man following epinephrine, insulin and surgical operations, *Proc. Soc. Exper. Biol. & Med.* **69:** 499–501 (Dec.) 1948.
27. Parsons, E. H.; Gildea, E. F.; Ronzoni, E., and Hulbert, S. A.: Comparative lymphocytic and biochemical responses of patients with schizophrenia and affective disorders to electroshock, insulin shock, and epinephrine, *Am. J. Psychiat.* **105:** 573–580 (Feb.) 1949.
28. Gabrilove, J. L.; Volterra, M.; Jacobs, M., and Soffer, L. J.: The effect of parenteral injection of epinephrine on leukocyte counts in normal subjects and in patients with Addison's disease, *Blood* **4:** 646–652 (May) 1949.
29. Long, C. N. H.: Personal communication.

THE NEUTRAL STEROIDS OF HUMAN URINE: QUANTITATIVE STUDIES IN VARIOUS CLINICAL CONDITIONS

SIDNEY LIONEL TOMPSETT*

From the Biochemical Department, Royal Infirmary, Edinburgh, Scotland

DATA on the levels of 17-ketosteroids in the urine of normal and diseased persons have been summarized by Dorfman (1). Isolation studies, chiefly in cases of adrenal cortical tumor and hyperplasia, have shown that urine may contain derivatives of the adrenal cortical hormones other than 17-ketosteroids. These may be classified as 20-ketosteroids or steroid alcohols. During the luteal phase of the menstrual cycle and in pregnancy, metabolites of progesterone such as pregnanediol and possibly pregnanolones (20-ketosteroids) are also found in urine.

Tompsett and Oastler (2) have separated the extract containing the urinary neutral steroids into "ketonic" and "nonketonic" fractions with Girard's reagent T and have determined the quantity of each gravimetrically. During late pregnancy the 17-ketosteroids formed only a small part of an increase in the "ketone" fraction which is probably due to pregnanolones. In a wide variety of other conditions the 17-ketosteroids, as determined by the Zimmermann reaction, formed the major part of the "ketonic" fraction and there was little evidence for the excretion of 20-ketosteroids in any appreciable quantity.

The "nonketonic" fraction contained measurable quantities of alcohols, *i.e.*, substances capable of forming hemi-succinates with succinic anhydride. Cholesterol was only a minor constituent.

A marked increase in the excretion of the "nonketonic" fraction as well as of 17-ketosteroids was observed in cases of nonspecific trauma. Pregnanediol as determined by the method of Astwood and Jones (3) formed an inappreciable part of the "nonketonic" fraction of male urine or of urine in cases of nonspecific trauma.

The "nonketonic" fraction contains a variable amount of nonalcoholic material but comparable values should be obtained by conversion of alcohols into hemi-succinates, their separation and their determination gravimetrically.

In a recent publication the writer (4) has described a technique whereby the actual content of nonketonic alcohols in the neutral steroid fraction was determined and the presence of 20-ketosteroids in any appreciable

Received for publication April 24, 1950.

* Present address: Clinical Laboratory, Royal Infirmary.

quantity detected or even roughly determined. The urinary neutral steroids were separated into "ketonic" and "nonketonic" fractions with Girard's reagent T. With succinic anhydride, alcohols in the "nonketonic" fraction were converted into hemi-succinates, which were separated and determined gravimetrically. Values for hemi-succinates were converted into free alcohols by use of the empirical factor 0.71. 17-Ketosteroids were determined colorimetrically in the "ketonic" fraction by means of the Zimmermann reaction, and the remainder converted into alcoholic hemi-succinates which were separated and determined gravimetrically. The actual content of alcoholic ketones was then calculated by use of the empirical factor 0.71. In the complete absence of 20-ketosteroids, alcoholic ketones should amount to about 85 to 90 per cent of the total 17-ketosteroids. The presence of 20-ketosteroids in appreciable quantity will be indicated by a marked increase in this percentage. Since 20-ketosteroids give only one eighth of the color of the corresponding quantity of 17-ketosteroid in the Zimmermann reaction, it should be possible then to calculate the quantity of the former, at least approximately.

It was also shown that only in pregnancy and possibly in adrenal cortical tumor were 20-ketosteroids present in any appreciable quantity. During late pregnancy, pregnanediol accounted for most of the "nonketonic" alcohols.

This investigation has now been extended to include a wider range of clinical conditions and especially those with a possible endocrine origin.

EXPERIMENTAL

The technique employed has been described in detail elsewhere (2, 4).

The complete 24-hour collection of urine was boiled with 0.1 volume of concentrated hydrochloric acid for ten minutes, cooled and extracted with toluol or ether. The extract was washed with 10 per cent aqueous sodium hydroxide and water and then evaporated to dryness in an all glass vacuum still. The dried residue was separated into "ketonic" and "nonketonic" fractions by means of Girard's reagent T. The 17-ketosteroid content of the "ketonic" fraction was determined by the technique of Holtorff and Koch (5). The alcohols of both fractions were converted into hemi-succinates with succinic anhydride in the presence of pyridine. Such were finally separated and determined gravimetrically. These techniques are essentially those described by Pincus and Pearlman (6).

In certain cases corticosteroids were also determined, using a modification of the method of Talbot, Saltzman, Wixom and Wolfe (7) (*see* Tompsett and Oastler (8)).

RESULTS

The results which are shown in Table 1 include values obtained in normal males and females, and in a variety of clinical conditions.

In general the values for "nonketonic" alcohols are of the same order as the 17-ketosteroids. Values for ketonic alcohols, with 2 exceptions, were

from 85 to 90 per cent of the values for 17-ketosteroids. Since such figures represent inappreciable concentrations of 20-ketosteroids, values for ketonic alcohols are therefore omitted. The urine from 1 case of virilism was examined on three occasions and the figures obtained suggest the presence of 20-ketosteroids in appreciable quantities.

Data from 2 cases of Cushing's disease (basophilism) are presented and in these it will be noted that although the excretion of corticoids is increased, values for 17-ketosteroids and "nonketonic" alcohols are within normal limits and there is no evidence for the presence of 20-ketosteroids in appreciable quantity.

The urines of 4 obese female subjects have also been examined. The figures obtained were similar to those of normal subjects. In 1 female subject with Addison's disease, the "nonketonic" alcohols were as low proportionately as the 17-ketosteroids.

DISCUSSION

The chemically determined values for the following groups of substances are believed to be related to certain endocrine functions: 1) corticosteroids, 2) pregnanediol, and 3) 17-ketosteroids.

In addition, the writer has in this and previous papers drawn attention to the presence in measurable quantities of "nonketonic" alcohols in the neutral steroid fraction of human urine. In the female such includes pregnanediol. In late pregnancy, pregnanediol accounts for the largest part of this fraction but in the nonpregnant female, other alcohols are obviously present in not negligible quantities. In the male as well as in the nonpregnant female, the "nonketonic" alcohols are excreted in amounts which are in general of the same order as the 17-ketosteroids. There is no proof as yet that these "nonketonic" alcohols originate from the adrenal cortex (or in the male, partially from the testes) but the writer believes that this is probably the case. It has been found that in conditions of hypoadrenocorticism or hyperadrenocorticism, the excretion of these "nonketonic" alcohols varies relatively to the 17-ketosteroid excretion.

The origin of such substances is of interest. Whether they have an origin similar to the 17-ketosteroids or represent a separate phase of adrenal cortical activity is as yet undetermined. Recently Schneider and Mason (9) have shown with in vitro experiments, that rabbit liver slices can convert 17-ketosteroids into "nonketonic" alcohols.

Although in general the excretion of total neutral steroids parallels the amount of 17-ketosteroids, that is, an increase or decrease in the one is accompanied by an increase or decrease in the other, there are occasions when such is not the case. In certain instances it has been noted that a low 17-ketosteroid excretion may be accompanied by a relatively high "nonketonic" alcohol excretion. This is particularly notable in 1 case of

virilism in which the analyses were carried out on three separate occasions. On two occasions, the excretion of 17-ketosteroids, and that of "nonketonic" alcohols were elevated similarly. On another occasion, whereas

TABLE 1. THE EXCRETION AND FRACTIONATION OF THE NEUTRAL STEROIDS OF HUMAN URINE

	Age (yrs.)	17-Ketosteroids (mg./24 hrs.)	"Nonketonic" alcohols (mg./24 hrs.)
		MALES	
Normal			
1	41	19.5	13.7
2	23	7.0	4.9
3	28	12.0	14.2
4	20	12.5	8.4
5	33	9.0	6.2
6	35	10.0	8.4
7	29	20.2	10.6
8	35	10.9	8.6
9	38	18.5	12.6
10	42	18.4	8.6
11	35	16.0	6.1
12	34	14.0	4.1
13	22	17.5	9.8
		FEMALES	
Normal			
1	12	1.3	11.1
2	38	8.0	14.2
3	35	4.7	16.1
4	28	9.4	8.6
5	53	5.4	8.2
6	51	8.3	6.1
7	21	9.0	10.6
8	24	8.1	4.2
9	28	11.6	12.2
10	32	8.8	5.0
Hirsutism			
11	21	17.9	16.1
12	60	6.0	8.1
13	25	6.3	10.6
14	21	16.8	18.1
15	28	17.0	27.6
16	21	29.4	36.6
17	35	6.4	14.6
18	36	16.0	14.4
19	41	19.3	6.7

TABLE 1—(*continued*)

	Age (yrs.)	17-Ketosteroids (mg./24 hrs.)	"Nonketonic" alcohols (mg./24 hrs.)
Hypertension			
20	51	4.6	2.1
21	37	8.1	1.6
22	52	2.3	3.9
23	47	8.0	10.6
24	61	2.7	10.5
25	52	3.7	10.8
26	56	5.7	10.6
Simmonds' disease			
27	38	0.8	1.6
28	39	0.5	1.2
Myxedema			
29	41	0.7	1.9
30	34	1.0	1.2
Paroxysmal hypertension			
31	33	1.0	17.2
Precocious puberty			
32	10	2.0	10.7
33	7	3.7	2.7
34	8½	3.8	6.1
Cushing's disease (basophilism)			
35	14	10.0 (8.9)*	6.5
		"Corticosteroids"—1.6 mg./24 hrs.	
36	8½	9.5 (8.7)	11.3
		"Corticoids"—1.1 mg./24 hrs.	
Virilism			
37	29	29.0	14.4
38	21	40.0 (46.1)	58.8
		35.0 (41.0)	41.6
		1.5 (14.4)	59.1
39	23	19.2	19.3
40	24	21.7	17.1

* Figures in brackets under the heading of 17-ketosteroids represent mg. of ketonic alcohols per 24 hours.

the excretion of "nonketonic" alcohols was elevated, the excretion of 17-ketosteroids was subnormal, although there was some evidence for the appreciable excretion of 20-ketosteroids.

The figures reported here throw little additional light on Cushing's disease (pituitary basophilism) or precocious puberty.

This study could probably be extended with some profit if urine could be examined from patients after treatment with such endocrine principles as ACTH or cortisone and correlated with other biochemical data.

SUMMARY

1. 17-Ketosteroids and "nonketonic" steroid alcohols have been determined in a variety of clinical conditions. The possibility of the presence of 20-ketosteroids (pregnanolones) in appreciable and measurable quantities has also been examined.

2. In the present series, the urinary content of "nonketonic" alcohols was generally of the same order as that of the 17-ketosteroids. The presence of 20-ketosteroids in appreciable quantity could be detected only in a single case of virilism.

3. Little additional information could be provided with respect to Cushing's disease (basophilism) or precocious puberty.

4. The relationship between the "nonketonic" alcohols and the 17-ketosteroids has been discussed with reference to their possible common origin from the suprarenal cortex.

REFERENCES

1. Dorfman, R. I.: Biochemistry of androgens, *in* The Hormones, New York, Academic Press, 1948, part 1, page 467.
2. Tompsett, S. L., and Oastler, E. G.: The neutral steroids of human urine: the significance of the various steroid fractions excreted in urine, *Glasgow Med. J.* **29:** 133, 1948.
3. Astwood, E. A., and Jones, G. E. S.: A simple method for the quantitative determination of pregnanediol in human urine, *J. Biol. Chem.* **37:** 397, 1941.
4. Tompsett, S. L.: The neutral steroids of human urine, *J. Endocrinol.* **6:** 192, 1949.
5. Holtorff, A. F., and Koch, F. C.: The colorimetric estimation of 17-ketosteroids and their applications to urine extracts, *J. Biol. Chem.* **135:** 377, 1940.
6. Pincus, G., and Pearlman, W. H.: Fractionation of neutral urinary steroids, *Endocrinology* **29:** 413, 1941.
7. Talbot, N. B.; Saltzman, A. H.; Wixom, R. L., and Wolfe, J. R.; Colorimetric assay of urinary corticosteroid-like substances, *J. Biol. Chem.* **160:** 535, 1945.
8. Tompsett, S. L., and Oastler, E. G.: The excretion of corticosteroids: the determination of the total free reducing ketosteroids in urine, *Glasgow Med. J.* **28:** 349, 1947.
9. Schneider, J. J., and Mason, H. L.: Studies on intermediary steroid metabolism. I. Isolation of Δ^5-androstene-3(β), 17(α)-diol and Δ^5-androstene-3(β), 16(β), 17(α)-triol following the incubation of dehydroisoandrosterone with surviving rabbit liver slices, *J. Biol. Chem.* **172:** 771, 1948. II. Compounds isolated following the incubation of androsterone and etiocholan-3(α)-ol-17-one with surviving rabbit liver slices, *J. Biol. Chem.* **175:** 231, 1948.

STUDIES ON HORMONAL CONTROL OF SERUM LIPID PARTITION IN MAN*†

DAVID ADLERSBERG, M.D., LOUIS E. SCHAEFER, M.D. AND STANLEY R. DRACHMAN, M.D.

From the Medical Services and the Department of Chemistry of the Mount Sinai Hospital, New York, N. Y.

CONSIDERABLE information is available concerning the hormonal control of carbohydrate and protein metabolism. The role of the pancreas, the thyroid, the anterior pituitary and the adrenal cortex in carbohydrate metabolism is well established by animal experiments and clinical experience. Similarly, the function of these endocrine organs, as well as of the gonads (particularly the testes), in controlling the metabolism of protein is well known.

In contrast, our knowledge of the hormonal control of lipid metabolism is limited, although experimental and clinical observations indicate that such a mechanism exists. It is established, for example, that hypothyroidism as well as hyperthyroidism alter lipid metabolism, especially the cholesterol content of the serum (1). Crude extracts of the anterior pituitary produce an increase in the lipid content of the liver and provoke ketonuria (2). Following pancreatectomy increased mobilization of fat occurs with an elevation of serum lipids and production of ketone bodies; these phenomena can be reversed by insulin administration (3).

It has long been suspected that the adrenal cortex plays an important role in lipid metabolism. Clinical observation suggests that hyperfunction of the adrenal cortex (Cushing's syndrome) produces hypercholesterolemia and an abnormal distribution of body fat, whereas adrenal insufficiency (Addison's disease) is associated with a low serum cholesterol and a loss of body fat (4). In the animal experiment, ketonuria and fatty infiltration of the liver produced by anterior pituitary extracts, by pancreatectomy, or by starvation can be prevented by adrenalectomy (5, 6). After adrenal homotransplantation or injection of adrenal cortical extracts, hypercholesterolemia is observed in rabbits (7). Administration of cortisone prevents loss of body fat in the adrenalectomized rat (8).

Received for publication June 30, 1950.

* Read at the Annual Meeting of the Association for the Study of Internal Secretions, San Francisco, June 23, 1950.

† This investigation was supported (in part) by a research grant from the Division of Research Grants and Fellowships, National Institutes of Health, United States Public Health Service.

Appreciation is expressed to Mrs. Rhoda Dritch for her technical assistance.

The availability of adrenal corticotropin and cortisone made it possible to analyze the effects of these hormones on the serum lipid partitions of patients who were receiving the hormones for therapeutic purposes.

METHOD

Observations on serum lipids as reported in the literature show marked variations. These differences are caused primarily by the different methods and techniques used. The determination of free and total cholesterol in our studies was carried out by the method of Sperry and Schoenheimer (9). Lipid phosphorus was analyzed by Sperry's modification (10) of the Fiske-Subbarow method, and the phospholipid was calculated by multiplying lipid phosphorus by 25 (expressed as mg. per 100 ml. of serum). Total lipids were determined by the method of Bloor (11). Various techniques have been used for the calculation of neutral fat in the serum. Peters and Van Slyke (12) estimated neutral fat by subtracting the amount of fatty acids combined with cholesterol and phospholipid from the value for total fatty acids. We estimated serum neutral fat by subtracting the figures for total cholesterol plus phospholipid from that for total fat. This simplified technique has also been used by others (13).

Blood specimens were always obtained from individuals fasting for at least nine hours. Since hemolysis may change serum lipid values, determinations were performed only on nonhemolyzed specimens.

RESULTS

To establish the normal range of serum cholesterol and phospholipid in our laboratory, 119 cholesterol and 121 phospholipid determinations were performed on a group of normal individuals. The average for the group was 211 mg. per 100 ml. for cholesterol, with a standard deviation of ± 30; for phospholipid the average was 249 mg. per 100 ml., with a standard deviation of ± 13. Although there was considerable fluctuation in the lipid levels, a certain constancy of serum cholesterol and phospholipid concentration was characteristic for a given individual over a period of many weeks, suggesting the existence of a regulatory mechanism. The average figures for this control group are in close agreement with those published by Peters and Van Slyke (12) and Kornerup (14).

The effect of cortisone administration on serum lipids

Cortisone acetate was administered to 18 patients over a period of from six to thirty-one days; the total dose per patient varied from 0.6 to 3.2 Gm. In 1 patient, two separate ten-day courses of treatment were given. The patients were suffering from a variety of conditions: 5 had lupus erythematosus disseminatus, 2 scleroderma, 2 rheumatoid arthritis, 3 leu-

kemia, and 1 each polyarteritis nodosa, nephrosis, Hodgkin's disease, generalized dermatitis, dermatomyositis, and acute rheumatic fever.

Cortisone was used in all patients for therapeutic purposes. Before the beginning of treatment and at various intervals during therapy the serum lipids were studied. When larger doses were administered the injections were usually given at six-hour intervals. When the doses were reduced in the course of therapy the hormone was administered every twelve hours. In all patients careful clinical observation was combined with detailed laboratory studies.

The essential data of the 18 patients are summarized in Tables 1, 2A, 2B, and 2C. In this group there were 9 males and 9 females. Their ages ranged from 13 to 60 years. Most of the patients were acutely ill, some almost moribund. There was a fatal termination in 3—in Patients 11 and 12 during cortisone therapy, and in Patient 5 five weeks after the cessation of cortisone treatment. In the other 16 instances (1 patient had two courses of treatment), the therapeutic response was as follows: excellent in 4 (2 lupus erythematosus disseminatus, 1 rheumatic fever); good in 7 (2 lupus erythematosus disseminatus, 2 rheumatoid arthritis, 1 each of dermatomyositis, generalized dermatitis, and Hodgkin's disease); moderate in 3 patients (2 scleroderma, 1 polyarteritis nodosa); and absent in 2 (1 acute and 1 chronic leukemia).

As can be seen in Table 2A, 14 of the 18 patients showed a gradual increase of total and esterified cholesterol, ranging from 2 per cent to 70 per cent for the total cholesterol, and from 1 per cent to 73 per cent for the esters. In 3 persons there was a decrease of cholesterol, and in 1 there was no change. The average change for all 18 patients was +19 per cent for the total and +23 per cent for esterified cholesterol. The elevation of total and esterified cholesterol was observed six to ten days after cortisone administration was begun, and, as a rule, the rise continued as long as the hormone was given. In 5 instances (Cases 1, 1A, 2, 3, 15) the total and esterified cholesterol continued to rise for a period of five to twenty days after discontinuation of cortisone administration. In all 4 instances in which a long follow-up after cessation of cortisone therapy was possible (Cases 1, 1A, 2, 5) the values eventually returned toward the normal.

A similar upward trend was observed in the behavior of the serum phospholipid (Table 2B). Fifteen of the 18 patients showed a gradual increase of the phospholipid level ranging from +15 per cent to +66 per cent; 2 patients showed a slight decrease; and in 1 case there was no change. The average change for the group was +24 per cent. Although in the majority of cases a parallelism was observed in the behavior of the cholesterol and phospholipid levels during cortisone therapy, occasionally a discrepancy was seen; *e.g.*, Patients 11 and 14 showed a decrease of

TABLE 1. CLINICAL DATA AND RESPONSE TO CORTISONE ACETATE TREATMENT IN 18 PATIENTS
(For duration of treatment and dose used, *see* Tables 2A, 2B, 2C)

Patient	Sex	Age (yrs.)	Diagnosis	Maximum temperature before therapy, °F.	ESR*	Blood Count			Bone marrow aspirations	Urine		Response to therapy and other findings	Change in body weight
						Hgb. Gm./100 cc.	RBC mill. per cu. mm.	WBC per cu. mm.		Albumin	Micro findings		
1. F.W.	M	13	Lupus erythematosus disseminatus	102	65	11.0	3.3	2,150	L.E.**	2+	Occ. wbc and rbc	Excellent. Complete symptomatic response; "butterfly" rash; congestive heart failure during treatment.	+4
1A. F.W.	M	13	Lupus erythematosus disseminatus	101					L.E.			Second course on Kempner diet. Excellent response after relapse following discontinuation of first course.	−5
2. S.M.	F	50	Polyarteritis nodosa	103	120	9.0	2.7	11,250	Neg.	1+	1-2 rbc	Moderate. Hemorrhagic rash on admission.	0
3. D.V.	F	21	Scleroderma	98	29	11.4	4.4	6,400	Neg.	0	0	Moderate. Very slight increase in motion of fingers.	+5
4. R.B.	M	34	Lupus erythematosus	101	130	8.0	3.0	4,200	L.E.	1+	0	Good.	+3
5. S.H.	F	50	Nephrosis	98	65	8.0	2.5	19,000	Neg.	4+	Many rbc	No response except slight loss of edema. Amyloidosis of kidneys, postmortem. Generalized dermatitis.	−6
6. D.A.	F	60	Rheumatoid arthritis	99	50	11.0	3.7	12,000	Neg.	0	0	Good. Able to walk for the first time in months.	+8
7. E.H.	M	10	Acute leukemia	103	55	7.0	2.0	6,000	Leuk.	0	0	None. Progressive downhill course.	
8. N.G.	M	5	Hodgkin's disease	101	50	8.0	3.4	9,500	Neg.	0	0	Good. Lymph nodes shrank and symptomatic improvement occurred.	+6
9. W.E.	M	51	Lupus erythematosus disseminatus	103	30	11.3	3.7	9,000	L.E.	0	0	Excellent, with relief of symptoms for the first time in a year.	+5
10. L.P.	F	36	Rheumatoid arthritis	100	55	10.5	4.0	9,000	Neg.	0	0	Good.	0
11. I.S.	F	22	Lupus erythematosus disseminatus	105	100	11.0	3.7	7,000	L.E.	2+	Granular casts; rbc	Moderate. Did not respond as well as others. Mental depression while on cortisone.	—
12. J.B.	M	55	Chronic lymphatic leukemia	104	100	10.5	3.5	25,000	Leuk.	1+	0	None as far as leukemia was concerned. Died of pulmonary infarction. Pulmonary tuberculosis discovered during treatment.	
13. M.M.	M	47	Generalized dermatitis	99	15	15.0	5.0	6,500	—	0	0	Good. Dermatitis resolved but angina developed while on cortisone. Dermatitis recurred later.	0
14. A.L.	M	60	Chronic lymphatic leukemia	102	120	5.0	1.5	7,000	Leuk.	0	0	None. Hemolytic anemia (severe); splenectomy performed. Died.	0
15. E.B.	F	49	Dermatomyositis	100	100	9.9	3.4	8,250	Neg.	1+	Occ. rbc	Good response in an acute case. Congestive heart failure developed but was easily controlled.	0
16. M.S.	M	45	Rheumatic fever	103	110	10.5	3.5	15,000	—	0	0	Excellent symptomatic and laboratory response. Discharged well, after 3 weeks.	
17. A.S.	F	47	Scleroderma	100	20	12.0	4.0	5,400		0	0	Moderate improvement of joint movements.	+3
18. R.C.	F	55	Lupus erythematosus disseminatus	100	17	12.5	4.2	5,700	L.E.	1+	0	Good.	+4

* ESR—Erythrocyte sedimentation rate in mm. per hour.
** L.E.—Lupus erythematosus cells.

TABLE 2A. CHANGES IN SERUM CHOLESTEROL IN 18 PATIENTS DURING CORTISONE ACETATE ADMINISTRATION

(For clinical data and response, *see* Table 1)

Patient	Cortisone		*Serum cholesterol**						
	No. of days given	Total dose, Gm.	Before cortisone	During cortisone administration (days)					Maximum change**
				1–5	6–10	11–15	16–20	21–25	26–30	
1. F.W.	10	1.2	192/121		247/161	284/176		293/200		+53
1A. F.W.	10	1.2	208/141	205/139	225/154				284/213	+36
2. S.M.	12	1.6	167/107			208/129	218			+31
3. D.V.	20	2.0	177/127	173/135			184/139		210	+19
4. R.B.	26	3.2	167/122		160/111		210		215/163	+29
5. S.H.	9	1.0	271/164		302/194					+11
6. D.A.	31	2.5	278/204			290/225			290/216	+ 4
7. E.H.	8	1.0	163/116		173/125					+ 6
8. N.G.	23	1.4	183			179/127		167/127		− 9
9. W.E.	16	1.8	211/137			248/187				+18
10. L.P.	23	1.8			155/112			200/156		+29
11. I.S.	19	2.7	212/151			188/122				−11
12. J.B.	6	0.6	153/94		188/125					+22
13. M.M.	6	0.8	500/372		510/377					+ 2
14. A.L.	9	0.9	131/84		100/62					−24
15. E.B.	25	2.3	239		286/215		300/221		346/258	+45
16. M.S.	7	0.7	155/108		234/168					+50
17. A.S.	18	1.5	272/187		274/197					0
18. R.C.	25	2.0	225/156		286/211		382/292			+70

* All data are expressed in mg. per 100 ml. The first cholesterol figure indicates total cholesterol and the second indicates esterified cholesterol.

** Expressed as percentage increase or decrease of the control value of total serum cholesterol.

TABLE 2B. CHANGES IN SERUM PHOSPHOLIPIDS IN 18 PATIENTS DURING CORTISONE ACETATE ADMINISTRATION

(For clinical data and response, *see* Table 1)

Patient	Cortisone		*Serum phospholipids**						
	No. of days given	Total dose, Gm.	Before cortisone	During cortisone administration (days)					Maximum change**
				1–5	6–10	11–15	16–20	21–25	26–30	
1. F.W.	10	1.2	240		354	369		398		+66
1A. F.W.	10	1.2	240	264	322			310		+34
2. S.M.	12	1.6	185			248	279			+51
3. D.V.	20	2.0	185	212			248		232	+34
4. R.B.	26	3.2	167		210		211		260	+57
5. S.H.	9	1.0	339		432					+27
6. D.A.	31	2.5	272			322	310		322	+18
7. E.H.	8	1.0	179		176					−10
8. N.G.	23	1.4	227			196		230		0
9. W.E.	16	1.8	271			322				+19
10. L.P.	23	1.8			217			268		+23
11. I.S.	19	2.7	232			260				+12
12. J.B.	6	0.6	188		238					+27
13. M.M.	6	0.8	421		485					+15
14. A.L.	9	0.9	205		252					+23
15. E.B.	25	2.3	331				365		403	+22
16. M.S.	7	0.7	215		273					+27
17. A.S.	18	1.5	288		267					− 7
18. R.C.	25	2.0	292		330		450			+54

* All data are expressed in mg. per 100 ml.

** Expressed as percentage increase or decrease of the control value.

TABLE 2C. CHANGES IN SERUM NEUTRAL FAT IN 18 PATIENTS DURING CORTISONE ACETATE ADMINISTRATION

(For clinical data and response, *see* Table 1)

Patient	Cortisone		*Serum neutral fat**						
	No. of days given	Total dose, Gm.	Before cortisone	During cortisone administration (days)					Maximum change**
				1–5	6–10	11–15	16–20	21–25	26–30	
1. F.W.	10	1.2			712	689		169		−76
1A. F.W.	10	1.2	342	404	316			216		−37
2. S.M.	12	1.6	356			257	149			−58
3. D.V.	20	2.0	273	285			138		58	−78
4. R.B.	26	3.2	277		213		109			−61
5. S.H.	9	1.0	680		506					−26
6. D.A.	31	2.5	177			273	136		68	−62
7. E.H.	8	1.0	208		144					−31
8. N.G.	23	1.4	280					209		−25
9. W.E.	16	1.8	241			120				−50
10. L.P.	23	1.8			301			287		− 5
11. I.S.	19	2.7								
12. J.B.	6	0.6								
13. M.M.	6	0.8								
14. A.L.	9	0.9	421		81					−81
15. E.B.	25	2.3								
16. M.S.	7	0.7	160		316					+97
17. A.S.	18	1.5	270		244					−10
18. R.C.	25	2.0	138				102			−26

* All data are expressed in mg. per 100 ml.

** Expressed as percentage increase or decrease of the control value.

serum cholesterol of −11 per cent and −24 per cent respectively, while the serum phospholipid rose +12 per cent and +23 per cent. It is of particular interest that the rise in cholesterol and phospholipid concentration occurred in Patients 1A and 5 even while they were maintained on a salt-free, fat-free, cholesterol-free diet (Kempner regimen).

In contrast to the behavior of serum cholesterol and phospholipid, there was a marked decrease in neutral fat during cortisone therapy (Table 2C). Of 15 patients, 14 showed this decrease, and in only 1 was there an elevation. It was of interest that the latter patient presented evidence of impairment of hepatic function. The average change of serum neutral fat for the entire group was −43 per cent. Peters and Van Slyke (12) stress the fact that the estimation of neutral fat suffers the cumulative errors of the cholesterol and lipid phosphorus analyses. The instability of this factor is enhanced by dietary causes to a much greater degree than the concentration of serum cholesterol or phospholipid.

Figure 1 illustrates the effect of cortisone on the serum lipids of a patient with lupus erythematosus disseminatus.

The effect of ACTH on serum lipids

In 16 patients (1 of whom received two courses), the effects of ACTH (Armour) administration on serum lipids were studied. This group of

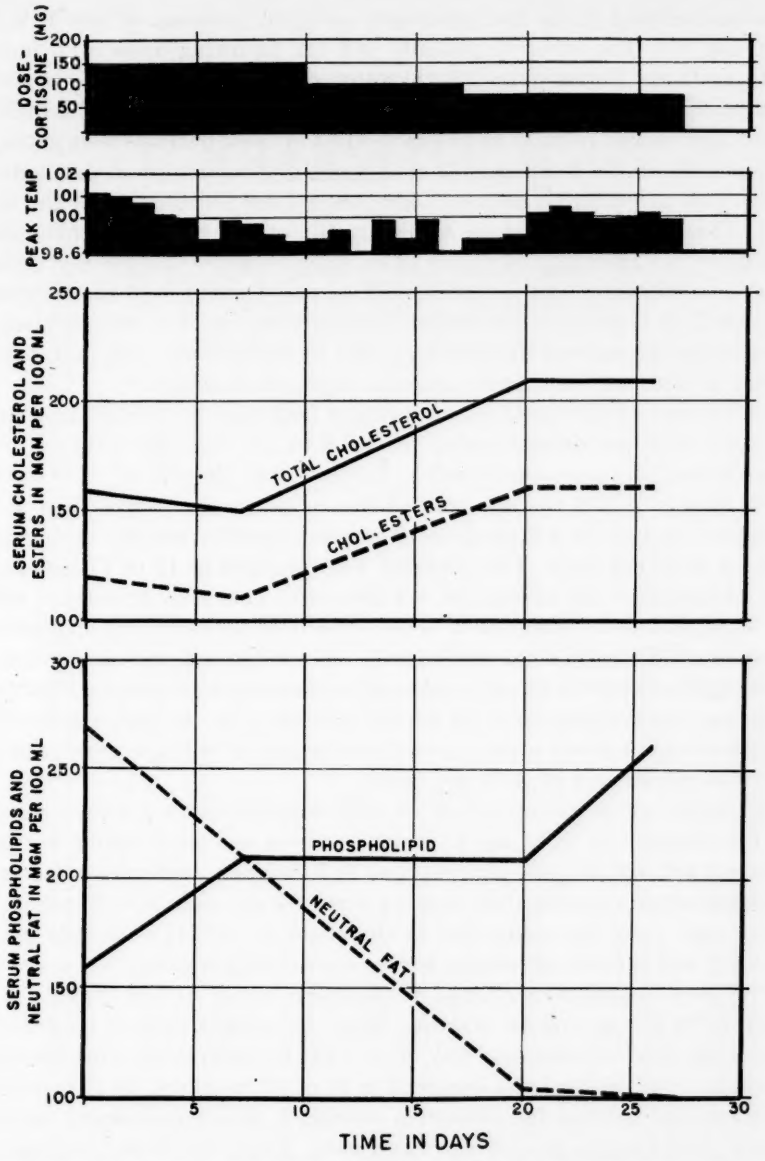

FIG. 1. Effect of cortisone administration on serum lipids in Patient 4 (lupus erythematosus disseminatus). A dose of 150 mg. was given for ten days, 100 mg. for seven days, and 75 mg. for the remainder of the therapy. The gradual increase of serum cholesterol, cholesterol esters, and phospholipid is accompanied by a decrease of serum neutral fat.

patients included 6 who had previously received cortisone (Cases 1, 2, 3, 4, 9, and 11). The other 10 patients had the following diseases: 5 lupus erythematosus disseminatus, 3 thyrotoxicosis, 1 gout, and 1 essential hypoproteinemia. The entire group consisted of 3 males and 13 females, whose ages ranged from 13 to 55 years. Most of these patients were acutely and severely ill. In 3 patients (2 with lupus erythematosus disseminatus, and 1 with polyarteritis nodosa) there was a fatal termination of the disease. The clinical response to ACTH in the others may be summarized as follows: in 4 instances there was an excellent response (2 lupus erythematosus disseminatus, 1 polyarteritis nodosa, and 1 gout); in 4 the response was good (4 lupus erythematosus disseminatus); in 2 it was moderate (1 lupus erythematosus disseminatus, and 1 scleroderma); and in 4 it was absent (3 thyrotoxicosis, and 1 essential hypoproteinemia).

ACTH was administered over a period of from nine to seventy days, and the total dose per patient varied from 0.8 to 3.8 Gm. The data for this group of patients are summarized in Tables 3, 4A, 4B, and 4C. The serum lipids during ACTH therapy showed less pronounced changes than with cortisone. Serum total cholesterol decreased slightly, usually during the first six to fifteen days. This decrease was observed in 13 of 17 instances and amounted to an average of −7 per cent. This is in agreement with the observations of Conn *et al.* (15). The drop in esterified cholesterol approximated that of total cholesterol (−7 per cent). Later in the course of therapy a moderate elevation of total and esterified cholesterol followed. This final rise occurred in 11 of the 17 instances. For the entire group the overall picture showed a rise in total cholesterol of +11 per cent, and in esterified cholesterol of +13 per cent.

Similarly, serum phospholipid showed considerable variations during ACTH therapy. In 10 of the 17 instances there was an elevation ranging from +1 per cent to +63 per cent; and in 7 instances there was a decrease of phospholipid concentration ranging from −9 per cent to −33 per cent. There was, then, no uniformity in the effect of ACTH on serum phospholipid, and the average change for the entire group was only +7 per cent.

The most conspicuous change of the lipids under ACTH therapy was observed in the neutral fat fraction. There occurred a drop of neutral fat during the sixth to twentieth day of ACTH administration. This decrease averaged −46 per cent and occurred in 13 of 16 instances. In 7 instances, however, the neutral fat showed a tendency to return toward control values even though ACTH was continued. Thus, the overall change in neutral fat, comparing the control with the final value, was −25 per cent.

Figure 2 illustrates the effect of ACTH upon serum lipids in a patient with lupus erythematosus disseminatus.

TABLE 3. CLINICAL DATA AND RESPONSE TO ACTH TREATMENT IN 16 PATIENTS. PATIENTS 1 TO 11 (TO WHOSE NUMBER A OR B ARE ADDED IN THIS SERIES) ARE IDENTICAL WITH THOSE OF TABLES 1 & 2 AND HAD PREVIOUS THERAPY WITH CORTISONE ACETATE. THEIR CLINICAL DATA ARE GIVEN IN TABLE 1.

Patient	Sex	Age (yrs.)	Diagnosis	Maximum temperature before therapy, °F.	ESR*	Blood Count			Bone marrow aspiration	Urine		Response to therapy and other findings	Change in body weight
						Hgb. Gm./100 cc.	RBC mill. per cu. mm.	WBC per cu. mm.		Albumin	Micro findings		
1B. F.W.	M	13	Lupus erythematosus disseminatus									Good, but not quite as dramatic as the two cortisone responses.	0
2A. S.M.	F	50	Polyarteritis nodosa									Excellent; better than on cortisone, but organic mental syndrome developed.	+ 5
2B. S.M.	F	50	Polyarteritis nodosa									Second ACTH course after relapse. Poor response. Died.	+7
3A. D.V.	F	21	Scleroderma									Moderate. Some slight increase over cortisone response. Facial fullness and hirsutism.	−13
4A. R.B.	M	34	Lupus erythematosus disseminatus									Good. Equal to cortisone. Pigmentation and facial fullness developed.	+ 7
9A. W.E.	M	51	Lupus erythematosus disseminatus									Good, but severe diabetes developed and required insulin.	−10
11A. I.S.	F	22	Lupus erythematosus disseminatus									Good, but died of intercurrent fungus infection.	+ 4
101. J.D.	F	13	Lupus erythematosus disseminatus	103	100	9.0	3.3	1,900	L.E.**	2+	rbc	Excellent, but died of convulsion on sixth day.	0
102. R.V.	F	20	Lupus erythematosus disseminatus	102	50	9.5	3.0	4,100	L.E.	1+	rbc	Moderate. Not as striking as in other lupus patients. Course was complicated by convulsions. Transitory congestive heart failure.	+10
103. D.E.	F	17	Hypoproteinemia	99	15	10.0	3.5	6,500	—	0	0	None. No change in serum proteins.	+14
104. S.L.	F	17	Lupus erythematosus disseminatus	101	92	10.4	3.2	4,600	L.E.	3+	rbc	Good symptomatic improvement. Not acutely ill initially.***	− 6
105. I.G.	F	55	Gout	98	50	14.0	4.7	21,000	—	0	0	Excellent joint response. Uricosuric effect of about 30 per cent.	− 4
106. C.B.	F	34	Lupus erythematosus disseminatus	104	110	7.5	2.0	2,100	L.E.	1+	rbc	Excellent clinical improvement.	0
107. N.M.	F	24	Lupus erythematosus disseminatus	103	100	8.0	3.1	3,600	L.E.	2+	rbc	Excellent clinical improvement.	+ 6
108. G.R.	F	18	Hyperthyroidism	98	3	12.5	4.0	6,350	—	0	0	None.	− 5
109. L.P.	F	32	Hyperthyroidism	99	8	13.0	4.5	5,050	—	0	0	None.	− 2
110. E.H.	F	28	Hyperthyroidism	98	2	13.9	5.0	6,550	—	0	0	None.	+ 1

* ESR—Erythrocyte sedimentation rate in mm. per hour.
** L.E.—Lupus erythematosus cells.
*** Patient had later a course of cortisone therapy.

TABLE 4A. CHANGES IN SERUM CHOLESTEROL IN 16 PATIENTS DURING ACTH ADMINISTRATION
(For clinical data and response, *see* Table 3)

Patient	ACTH		*Serum cholesterol**							
	No. of days given	Total dose, Gm.	Before ACTH	During ACTH administration (days)						Final change***
				1–5	6–10	11–15	16–20	21–25	26–30	Over** 30 days	
1B. F.M.	30	1.3	246/164			271/200		284/218	262/196		+ 7
2A. S.M.	29	2.1	218	191/137		194/137		267/192	270		+24
2B. S.M.	22	1.8	174/125			212/148	180/106				+22
3A. D.V.	18	0.9			210		198/149				− 6
4A. R.B.	54	1.7			215/163				198/149	235/179 (54)	+ 9
9A. W.E.	60	2.0	248/187			230/185			167/113	242/167 (61)	− 2
11A. I.S.	17	1.4		188/122	205/149			199/145			+ 6
101. J.D.	9	0.8	289/182	278/191							− 4
102. R.V.	70	3.8	132/85		132/87	111/59		96/66	85/57	170/116 (65)	+30
103. D.E.	12	1.4	175			156/115	263/204				+50
104. S.L.	49	2.5	264/175			295	290/212	250/173		323/229 (44)	+23
105. I.G.	20	1.0	284/201		234/165						−18
106. C.B.	43	2.3	134/69		120/59	167/113	149/113		157/117	201/139 (33)	+50
107. N.M.	30	2.1	180/111	206/140	255/194		164/204	250/184			+39
108. G.R.	10	1.0	143/107		123/93	154/119					+ 8
109. L.P.	10	1.0	137/104		114/84	132/88					− 4
110. A.H.	10	1.0	204/162		167/132	175/143					−14

* All data are expressed in mg. per 100 ml. The first cholesterol figure indicates total cholesterol, and the second indicates esterified cholesterol.

** The day is indicated by the figure in parentheses.

*** Expressed as percentage increase or decrease of the control value of total serum cholesterol.

TABLE 4B. CHANGES IN SERUM PHOSPHOLIPIDS IN 16 PATIENTS DURING ACTH ADMINISTRATION

(For clinical data and response, *see* Table 3)

Patient	ACTH		*Serum phospholipids**								
	No. of days given	Total dose, Gm.	Before ACTH	During ACTH administration (days)						Over** 30 days	Maximum*** change
				1–5	6–10	11–15	16–20	21–25	26–30		
1B. F.W.	30	1.3	299			350		280	248		+17
2A. S.M.	29	2.1	279			280		272	304		+ 8
2B. S.M.	22	1.8	222	262		310	267				+40
3A. D.V.	18	0.9			232		244				+ 5
4A. R.B.	54	1.7			260				222	235 (54)	−10
9A. W.E.	60	2.0	271			252			252	331 (61)	+31
11A. I.S.	17	1.4		260	256			205			−21
101. J.D.	9	0.8	349	352							+ 1
102. R.V.	70	3.8	174		191	285		130	109	234 (65)	+63
103. D.E.	12	1.4	205			232	268				+31
104. S.L.	49	2.5	285			311	291	313		348 (44)	+22
105. I.G.	20	1.0	307		276						−10
106. C.B.	43	2.3	196		230	222	218		228	272 (33)	+39
107. N.M.	30	2.1	273	264	248		260	272			− 9
108. G.R.	10	1.0	209		179	184					−14
109. L.P.	10	1.0	198		133	169					−33
110. A.H.	10	1.0	218		209	176					−19

* All data are expressed in mg. per 100 ml.

** The day is indicated by the number in parentheses.

*** Expressed as percentage increase or decrease of the control value.

TABLE 4C. CHANGES IN SERUM NEUTRAL FAT IN 16 PATIENTS DURING ACTH ADMINISTRATION

(For clinical data and response, *see* Table 3)

Patient	ACTH		*Serum neutral fat**									
	No. of days given	Total dose, Gm.	Before ACTH	During ACTH administration (days)						Over** 30 days	Initial change***	Overall change***
				1–5	6–10	11–15	16–20	21–25	26–30			
1B. F.W.	30	1.3	470			379		286	410		−39	−13
2A. S.M.	29	2.1	149			154		124	202		−17	+39
2B. S.M.	22	1.8		167		118					−29	−29
3A. D.V.	18	0.9										
4A. R.B.	54	1.7							167	56 (54)	−66	−66
9A. W.E.	60	2.0				253			271	112 (61)	+ 6	−56
11A. I.S.	17	1.4		402	139			46			−88	−88
101. J.D.	9	0.8	395	493							+25	+25
102. R.V.	70	3.8	177		57	21		14	56	232 (65)	−92	+31
103. D.E.	12	1.4	319			27					−91	−91
104. S.L.	49	2.5	341			301	239	110		241 (44)	−68	−29
105. I.G.	20	1.0	449		280						−38	−38
106. C.B.	43	2.3	87		143	301	53		268	242 (33)	−39	+178
107. N.M.	30	2.1	194	50	57		101	158			−74	−19
108. G.R.	10	1.0	208		88	252					−58	+21
109. L.P.	10	1.0	165		193	209					+27	+27
110. A.H.	10	1.0	303		149	99					−67	−67

* All data are expressed in mg. per 100 ml.

** The day is indicated by the number in parentheses.

*** Expressed as percentage increase or decrease of the control value.

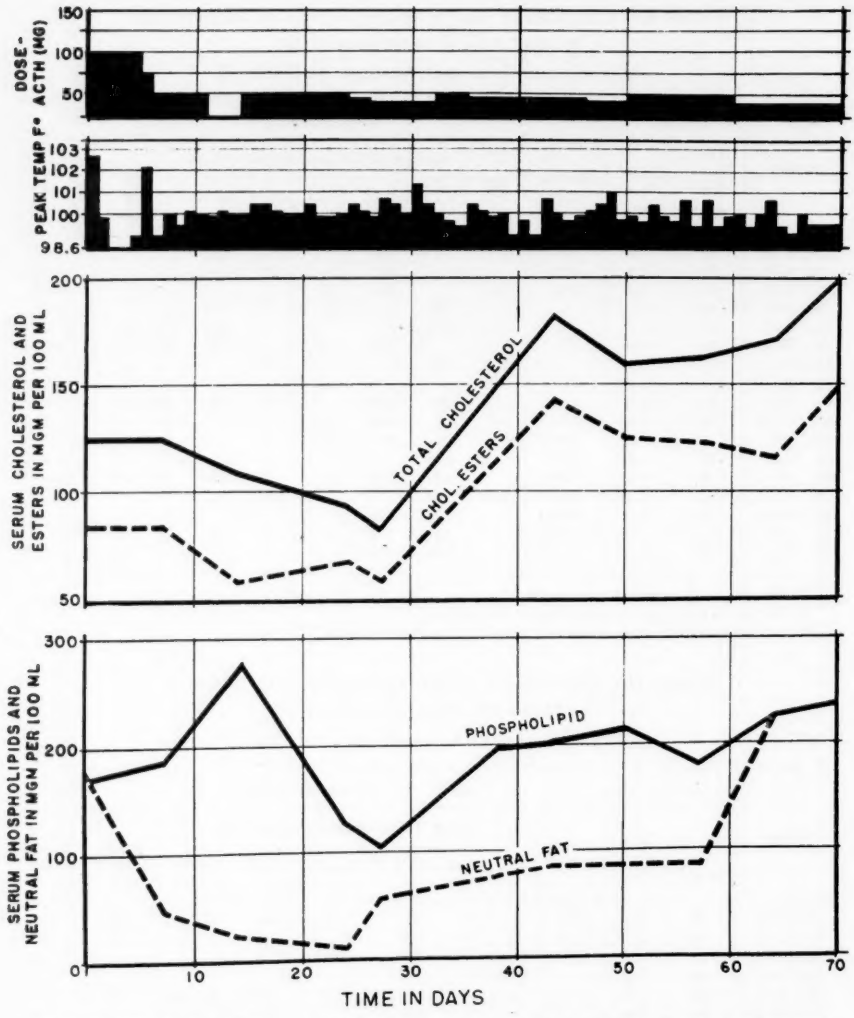

FIG. 2. Effect of ACTH administration on serum lipids in Patient 102 (lupus erythematosus disseminatus). The original dose of 100 mg. daily was maintained for five days, was then reduced to 75 mg. for one day, and then to 50 mg. for five days. After discontinuation of therapy for three days, the patient was then maintained on 40–50 mg. daily for fifty-six days. There is an initial decrease of total and esterified cholesterol which is followed by an increase of both fractions later in the course of treatment. Serum neutral fat shows an almost parallel decrease and then gradually returns toward normal. Serum phospholipid fluctuates around the normal range.

DISCUSSION

The concept of hormonal regulation of lipid metabolism and especially of the serum lipid partition receives additional support from these observations. The relative constancy of the lipid levels for the normal individual is emphasized.

The changes of the serum lipid partition produced by cortisone consisted of progressive elevation of total and esterified cholesterol as well as of phospholipid, and a decrease in neutral fat. After discontinuance of cortisone administration a return toward the control values of the serum lipid partition was observed. Thus, one of the adrenal hormones, cortisone, has been found to affect the concentration of the serum lipids, indicating a possible role of the adrenal cortex in lipid metabolism.

As with cortisone, there was an elevation of serum cholesterol and a drop in serum neutral fat during ACTH therapy. Unlike cortisone, however, ACTH produced an initial decrease of both total and esterified cholesterol; little, if any, change in serum phospholipid; and, after the initial fall, a trend toward a return to the control value for neutral fat even with continued hormone administration. These observations appear to substantiate previous work which has shown that anterior pituitary extracts affect the lipids (2).

The difference between the effects of cortisone and ACTH on the pattern of serum lipids may be explained by the differences in points of attack of these substances. Evidence is available that the adrenal cortex of man, when stimulated by ACTH, secretes various cortical hormones and probably more Compound F than Compound E (cortisone) (16). Further, there is reason to believe that serum cholesterol may furnish the material for the synthesis of cortical steroid hormones (15). Administration of ACTH produces a sudden demand for precursors of cortical hormones, one of which is probably cholesterol. This factor interferes with the concentration of cholesterol in the tissues and in the serum and in this way affects the concentrations of the other serum lipids.

Clinical and experimental observations indicate that prolonged administration of large doses of cortisone, results in atrophy of the adrenal cortex (16, 17). In addition, the possibility of anti-ACTH hormone production must be considered (18). The tendency of serum neutral fat to return toward normal levels despite continued administration of the hormone, as well as the transitory character of the serum cholesterol drop under ACTH, which is later followed by a moderate elevation, might be explained on this basis.

Of interest was the observation that the fasting sera of approximately one half of the patients treated with cortisone, and one quarter of those treated with ACTH became turbid or opaque despite the marked lowering

of the levels of serum neutral fat. This observation may indicate the production of a lipid which is less soluble or contains larger particles than normal, or a change in the conjugation of the lipids with the proteins. Recent studies have shown that the lipids of the blood and tissues are in combination with other substances rather than in a free state. About three quarters of the serum lipid is bound in the well-defined beta-lipoprotein (19). The hormonal changes produced by cortisone or ACTH administration may easily upset the ratio between free and conjugated lipids, especially since a change of the globulin fractions has been observed in electrophoretic studies (20).

Any attempts to explain further the changes of serum lipid levels caused by cortisone or ACTH would be speculative. Whether the synthesis of cholesterol and/or phospholipid is enhanced, or the intestinal absorption of these substances accelerated, or their deposition in the liver and other tissues affected cannot be stated. The apposition of tissue fat is apparently enhanced by cortisone and ACTH (8, 21). Studies will be necessary to clarify the involved hormonal (thyroid, pancreatic, etc.) and enzymatic changes produced by the adrenal cortex in lipid metabolism. It must be stressed that our observations were made on patients with a variety of diseases. The conclusions drawn from these results, therefore, do not necessarily apply to normal individuals.

The probable role of the adrenal cortex in lipid metabolism may explain some clinical observations made in diseases of the adrenal. The increased deposition of tissue fat, especially in the face, of patients with Cushing's syndrome, and the presence of abdominal striae in this disease may be explained by the increased production of hormones affecting fat metabolism. Similarly, the round contour of the face and the "buffalo hump" of the cervico-thoracic junction observed during cortisone and ACTH therapy are probably caused by the same mechanism. On the other hand, the striking wasting of fat tissue in Addison's disease as well as in adrenalectomized animals becomes understandable if one considers the diminished or absent production of these hormones. Similarly, the higher serum cholesterol levels observed in patients with Cushing's syndrome and the diminished concentration of this substance in patients with Addison's disease are possibly explained by the hyperproduction or hypoproduction of such hormones.

Although not generally confirmed, the impression is that Cushing's disease is associated with extensive atherosclerosis (22). Recently, importance has been ascribed to a disturbed lipid metabolism in the genesis of atherosclerosis in familial xanthomatosis (23, 24), and in familial atherosclerosis of young age (25). The alteration of serum lipids in Cush-

ing's syndrome resembles, to a certain extent, that found in the above-mentioned conditions. The higher incidence of atherosclerosis in Cushing's syndrome could thus be related to the overproduction of hormones of the adrenal cortex concerned with lipid metabolism. Additional observations are necessary to establish the importance of the cortical hormones in pathologic conditions affecting the adrenal cortex, as well as in other diseases characterized by abnormalities of the serum lipid pattern, *e.g.*, diseases of the thyroid.

These observations concerning the probable role of the adrenal cortex in lipid metabolism shed additional light on well-established experimental phenomena. The prevention of fatty infiltration of the liver by adrenalectomy (5, 6), and the marked hypercholesterolemia observed in animals after adrenal homotransplantation (7) may be explained on this basis. Similarly, the prevention of loss of body fat in the adrenalectomized animal by the administration of cortisone (8) becomes more clear.

SUMMARY

The serum lipid partition was studied in a group of 18 patients who were treated with cortisone acetate over a period of from six to thirty-one days, with a total dose varying from 0.6 to 3.2 Gm. per person. These patients had a variety of clinical conditions including lupus erythematosus disseminatus, scleroderma, rheumatoid arthritis, leukemia, polyarteritis nodosa, nephrosis, Hodgkin's disease, generalized dermatitis, dermatomyositis, and acute rheumatic fever. Most of the patients were severely ill, and there was a fatal termination in 3. During cortisone administration a gradual increase of total and esterified cholesterol was observed, which averaged +19 per cent for total cholesterol and +23 per cent for the esters. A similar trend was observed in the serum phospholipid; the total change for the group was +24 per cent. The rise in serum cholesterol and phospholipid occurred in 2 instances on a salt-free, fat-free, cholesterol-free diet. In contrast, there was a marked decrease in serum neutral fat, which averaged −43 per cent for the entire group.

In 16 patients the effect of ACTH administration was studied. This hormone was given over a period of from nine to seventy days, in a total dose varying from 0.8 to 3.8 Gm. per patient. The serum lipids showed less conspicuous changes than with cortisone. Serum cholesterol usually decreased slightly during the first six to fifteen days by an average of −7 per cent; a moderate elevation of this fraction then followed, the rise in total cholesterol averaging +11 per cent, and that of the esters +13 per cent. The serum phospholipid showed no uniform change under ACTH, the average being a slight increase (+7 per cent). The neutral fat of the serum showed

a maximum drop during the sixth to the twentieth day which averaged −46 per cent, and showed later a tendency to return toward control values. The overall change in neutral fat was −25 per cent.

A group of normal individuals was studied to establish normal values for our laboratory. A total of 119 cholesterol and 121 phospholipid determinations revealed an average of 211 mg. per 100 ml. ±30, for serum cholesterol. The serum phospholipid averaged 249 mg. ±13.

REFERENCES

1. Peters, J. P., and Man, E. B.: The significance of serum cholesterol in thyroid disease, *J. Clin. Investigation* **29:** 1, 1950.
2. Best, C. H., and Campbell, J.: Anterior pituitary extracts and liver fat, *J. Physiol.* **86:** 190, 1936.
   Foglia, V. G., and Mazzocco, P.: Action de l'estrait antéro-hypophysaire sur la graisse hépatique et musculaire, *Compt. rend. soc. de biol.* **127:** 150, 1938.
   Neufeld, A. H., and Collip, J. B.: The effect of pituitary extracts on ketonuria, fat content, and fat distribution in the liver and tissues of mice, *Endocrinology* **25:** 768, 1939.
3. Bloor, W. R.; Gillette, E. M., and James, M. S.: Fat metabolism in diabetes. I. The blood lipids in experimental diabetes, *J. Biol. Chem.* **75:** 61, 1927.
   Lichtman, A. L.: Fatty acids and glucose in the blood of depancreatized dogs, *J. Biol. Chem.* **120:** 35, 1937.
4. Selye, H.: Textbook of Endocrinology. Acta Endocrinologica, Montreal, Université de Montreal, 1947, (hypocorticoidism, p. 145; hypercorticoidism, p. 167).
5. MacKay, E. M., and Barnes, R. H.: The effect of adrenalectomy on liver fat in fasting and after the administration of anterior pituitary extracts, *Am. J. Physiol.* **118:** 525, 1937.
6. Fry, E. G.: The effect of adrenalectomy and thyroidectomy on ketonuria and liver fat content of the albino rat following injections of anterior pituitary extract, *Endocrinology* **21:** 283, 1937.
7. Hoffmeyer, J.: Influence of adrenal cortex on cholesterol content in rabbit serum, *Acta. physiol. Scandinav.* **10:** 31, 1945.
8. Stoerk, H.: Personal communication.
9. Sperry, W. M., and Schoenheimer, R.: A comparison of serum, heparinized plasma and, oxalated plasma in regard to cholesterol content, *J. Biol. Chem.* **110:** 655, 1935.
10. Sperry, W. M.: Electrophotometric microdetermination of phosphorus in lipide extracts, *Indust. & Engin. Chem.* anal. ed., **14:** 88, 1942.
11. Bloor, W. R.: Studies on blood fat. I. Variations in the fat content of the blood under approximately normal conditions, *J. Biol. Chem.* **19:** 1, 1914.
12. Peters, J. P., and Van Slyke, D. D.: Quantitative Clinical Chemistry. Interpretations, ed. 2., Baltimore, The Williams and Wilkins Co., 1946, vol. 1, chapter 5, p. 470.
13. Ahrens, E. H., Jr., and Kunkel, H. J.: The stabilization of serum lipid emulsions by serum phospholipids, *J. Exper. Med.* **19:** 409, 1949.
14. Kornerup, V.: Concentration of cholesterol, total fat and phospholipid in serum of normal man: report of a study with special reference to sex, age and constitutional type, *Arch. Int. Med.* **85:** 398, 1950.
15. Conn, J. W., and Vogel, W. C.: Effects of prolonged adrenal cortical stimulation

upon free and esterified serum cholesterol in normal men, (abstract) *J. Clin. Endocrinol.* **9:** 656, 1949.

16. Sprague, R. G.; Power, M. P.; Mason, H. L.; Albert, A.; Mathieson, D. R.; Hench, P. S.; Kendall, E. C.; Slocumb, C. H., and Polley, H. F.: Observations on the physiologic effects of cortisone and ACTH in man, *Arch. Int. Med.* **85:** 199, 1950.
17. Antopol, W.: Anatomic changes produced in mice treated with excessive doses of cortisone, *Proc. Soc. Exper. Biol. & Med.* **73:** 262, 1950.
18. Gordon, G. L.: The development of a refractory state to adrenocorticotrophic hormone, *Endocrinology* **45:** 571, 1949.
19. Oncley, J. L.; Gurd, F. R. N., and Melin, M.: Preparation and properties of serum and plasma proteins. XXV. Composition and properties of human serum beta-lipoprotein, *J. Am. Chem. Soc.* **72:** 458, 1950.
20. Reiner, M.: The effect of cortisone and ACTH on serum protein in disseminated lupus erythematosus, *Proc. Soc. Exper. Biol. & Med.* (in press).
21. Baker, B. L.; Ingle, D. J.; Li, C. H., and Evans, H. M.: The effect on liver structure of treatment with adrenal corticotropin under varied dietary conditions, *Am. J. Anat.* **82:** 75, 1948.
22. Heinbecker, P., and Pfeiffenberger, M., Jr.: Further clinical and experimental studies on the pathogenesis of Cushing's syndrome, *Am. J. Med.* **9:** 3, 1950.
23. Adlersberg, D.; Parets, A. D., and Boas, E. P.: Genetics of atherosclerosis: studies of families with xanthoma and unselected patients with coronary artery disease under the age of fifty years, *J. A. M. A.* **141:** 246, 1949.
24. Wilkinson, C. F., Jr.; Hand, E. A., and Fliegelman, M. T.: Essential familial hypercholesterolemia, *Ann. Int. Med.* **29:** 676, 1948.
25. Boas, E. P.; Parets, A. D., and Adlersberg, D.: Hereditary disturbance of cholesterol metabolism: a factor in the genesis of atherosclerosis, *Am. Heart J.* **35:** 611, 1948.

THE EXCRETION OF ACID PHOSPHATASE AS A MEASURE OF PROSTATIC DEVELOPMENT DURING PUBESCENCE

LELAND C. CLARK, JR., PH.D., ELIZABETH BECK, M.S. AND HASKELL THOMPSON, M.S.

From the Fels Research Institute for the Study of Human Development, Antioch College, Yellow Springs, Ohio

A RELATIVELY simple and reliable biochemical measure of physiologic pubescence would be a valuable adjunct to various anthropologic criteria of sexual development. Since the prostate is an organ which is very sensitive to stimulation by androgens (1, 2) a chemical procedure for assaying its secretory power was studied as a means of measuring the beginning of pubescence in the male. Since females have no, or only residual prostatic tissue (3), they can be regarded for the present purpose as a control group. It has been established by various means (4, 5, 6) that the enzyme, acid phosphatase, excreted in the urine, is derived mainly from the prostate.

The present paper deals with the excretion of this enzyme by a group of children between the ages of 2 and 18 years, and by a small group of adults.[1]

METHOD

Postabsorptive urine samples

Urine samples for acid phosphatase determination were collected by the subjects in the morning directly after they had undergone a basal metabolism test and before breakfast. A timed interval of urine collection was arranged by having the subject discard the first morning sample and collect that formed during a subsequent timed interval of between forty and two hundred minutes. All samples having volumes below 250 ml. were diluted to this volume.

The samples were assayed for creatinine (7) and for acid phosphatase by the method of Huggins (8) using sodium phenolphthalein phosphate as the substrate. The enzyme activity is expressed as phenolphthalein units according to Huggins.

24-Hour urine samples

Twenty-four hour urine samples were collected in the presence of dilute HCl and stored in the cold until assayed. All samples were analyzed for creatinine and collections repeated if necessary. The urine was hydrolyzed

Received for publication June 14, 1950.

[1] The subjects were obtained from families regularly enrolled in the Institute's research program.

and extracted by the CCl_4 method of Talbot (9) using the Wolfe-Hershberg extractor (10). The extracts were assayed by the method of Koch (11) using a sixty-minute development time. The ketonic fraction (12) of these extracts was also assayed on many of these samples but for the present purposes, the results for the "crude" extracts only are reported.

RESULTS AND DISCUSSION

The data obtained, along with the statistical expression of the variability encountered, are presented in Table 1.

TABLE 1. EXCRETION OF ACID PHOSPHATASE BY CHILDREN—NORMS

Age (months)	Sex	N*	Acid phosphatase excretion							
			Total units		Units/ml.		Units/mg. of creatinine		Units/min.	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
72–95	M	21	15.2	5.78	0.055	0.020	0.283	0.065	0.116	0.037
	F	14	13.9	6.61	0.054	0.027	0.316	0.121	0.098	0.057
	T	35	14.7	6.16	0.055	0.022	0.299	0.100	0.109	0.047
96–119	M	18	21.6	12.10	0.068	0.037	0.247	0.107	0.139	0.057
	F	18	14.8	9.08	0.056	0.036	0.250	0.109	0.100	0.039
	T	36	18.2	11.20	0.062	0.037	0.248	0.109	0.119	0.054
120–143	M	14	19.4	6.63	0.064	0.022	0.226	0.076	0.141	0.044
	F	20	14.6	6.50	0.052	0.024	0.209	0.070	0.099	0.044
	T	34	16.6	6.97	0.057	0.024	0.216	0.071	0.117	0.048
144–167	M	20	33.8	26.70	0.113	0.088	0.305	0.186	0.259	0.226
	F	22	24.6	9.18	0.081	0.028	0.238	0.067	0.170	0.078
	T	42	29.0	20.10	0.096	0.066	0.271	0.142	0.213	0.171
168–191	M	18	51.8	24.80	0.180	0.099	0.400	0.256	0.441	0.264
	F	15	25.5	5.64	0.083	0.020	0.235	0.046	0.176	0.041
	T	33	39.8	22.80	0.136	0.089	0.326	0.209	0.321	0.237
192–215	M	12	62.2	24.20	0.176	0.092	0.342	0.096	0.449	0.167
	F	10	27.5	8.96	0.090	0.040	0.240	0.023	0.194	0.068
	T	22	46.4	25.60	0.137	0.085	0.296	0.089	0.333	0.183
Total	M	103	32.4	25.00	0.106	0.084	0.302	0.166	0.247	0.212
	F	99	19.6	9.66	0.068	0.033	0.245	0.088	0.137	0.068
	T	202	26.1	20.10	0.087	0.068	0.273	0.135	0.193	0.169

* N = Number of determinations.

The most meaningful quantitative expression of excretion of the enzyme is, theoretically, units/time interval since when expressed as units/mg. of creatinine, the differences between age groups is diminished due to the gradually increasing *rate* of excretion of creatinine with age. However, an error in timing of the fasting sample would seriously affect the results. Therefore, in order to determine if any significant errors in timing had occurred, the data were recalculated from units/mg. of creatinine to units/min. by multiplying the units/mg. of creatinine by the creatinine excretion rate (as mg. per minute) obtained from independent analyses[2] of 24-hour urine samples from children. This information, presented in Table 2, reveals a striking concordance and indicates that possible errors in timing have not influenced the mean rate calculations. The fact that the coefficient of variation of the phosphatase/creatinine is less (S.D./M = 49.5 per cent) than the phosphatase/min. (S.D./N = 87.5 per cent) reveals, however, that certain individual errors in timing were made.

TABLE 2. ACCURACY OF DETERMINATION OF ACID PHOSPHATASE AS CALCULATED FROM INDEPENDENTLY OBTAINED CREATININE ANALYSES

Age group (yrs.)	Creatinine mg. per 24 hrs.	Creatinine mg. per minute	Phosphatase* per mg. creatinine units/mg.	Phosphatase units excreted per minute: Calculated units/min.	Phosphatase units excreted per minute: Found* units/min.
6–7	517	0.36	0.30	0.11	0.11
8–9	639	0.44	0.25	0.11	0.12
10–11	809	0.56	0.22	0.12	0.12
12–13	997	0.69	0.27	0.19	0.21
14–15	1340	0.93	0.33	0.31	0.32
16–17	1584	1.10	0.30	0.33	0.33
Total	955	0.66	0.27	0.18	0.19

* From Table 1.

In Table 1, it can be seen that, irrespective of the method of calculation, males excrete the enzyme at a higher rate than females after the age of about 12 years. The mean phosphatase excretion by children between the ages of 2 and 6 years was found to be 0.076 for males (N = 18) and 0.077 units per minute for females (N = 12), with a standard deviation of 0.034. Figure 1 illustrates the rapid increase found at pubescence in males and

[2] These creatinine normative data represent the analysis of about 500 24-hour urine samples from children in various age groups. (Unpublished results from this laboratory.)

TABLE 3. EXCRETION OF ACID PHOSPHATASE BY ADULTS

Age (yrs.)	Sex	N	Acid phosphatase excretion							
			Total units		Units/ml.		Units/mg. creatinine		Units/min.	
			M	S.D.	M	S.D.	M	S.D.	M	S.D.
29–58 M=44.4	M	12	66.1	41.0	0.284	0.16	0.914	0.53	0.650	0.38
20–42 M=33.6	Fp*	70	21.6	17.0	0.078	0.06	0.282	0.17	0.175	0.11
24–42 M=37.3	F**	11	29.1	9.7	0.102	0.05	0.294	0.11	0.206	0.11

*Fp = Pregnant women, 3–10 lunar months.
** F = Samples from the Fp cases at 1 year after parturition.

Table 3 indicates that males finally reach values about 3.5 times those of females. Similar results were reported by Huggins for a series of adults. It can be added, parenthetically, that pregnancy does not affect the excretion of acid phosphatase.

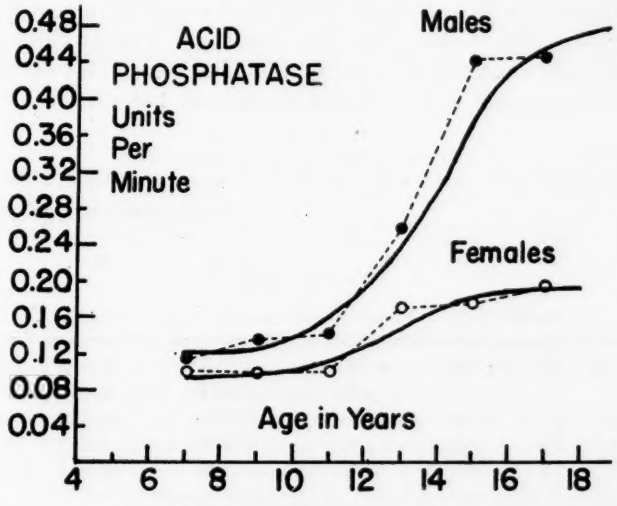

FIG. 1. Acid phosphatase excretion related to age.

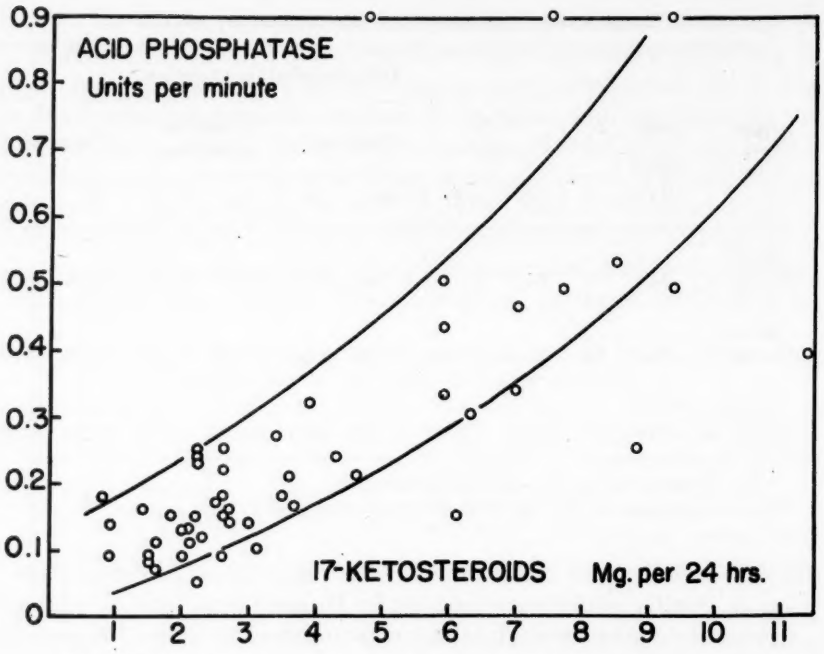

FIG. 2. Relation between acid phosphatase and 17-ketosteroid excretion.

From Figure 2, which compares the rates of excretion of acid phosphatase and 17-ketosteroids, it can be seen that although there is a general relationship, individual variation is considerable. The correlation coefficient (13) between ketosteroids and acid phosphatase is 0.48 ($N = 78$, $rxy = 0.48$).

TABLE 4. INDIVIDUAL VARIABILITY

Subject	Sex	Age (yrs.)	N	Phosphatase (units per minute)		
				Mean	S.D.	$\frac{\text{S.D.}}{\text{M}} \times 100$
A	M	2	9	0.065	0.035	54.0
B	F	1.5	6	0.064	0.028	43.7
C	F	18	6	0.154	0.039	25.3
D	Fp	38	8	0.235	0.089	37.9
E	Fp	31	6	0.225	0.051	22.6
Mean				0.149	0.048	36.7

This indicates that individual prostates do not respond to the same level of circulating androgen, as reflected by urinary 17-ketosteroid measurements. For example, one child showed a tenfold increase in acid phosphatase in one year during which time his ketosteroid excretion increased from 4.5 to 4.9 mg. per twenty-four hours, whereas another child doubled the acid phosphatase excretion rate while his ketosteroid excretion increased from 5.4 to 6.8 mg. per twenty-four hours.

It must also be realized that certain environmental factors (14) affect the rate of excretion of acid phosphatase and thus also add to the variability. This variability (Table 4), expressed as the coefficient of variation is not much less than that obtained in the entire age study (Table 1).

SUMMARY

Urinary acid phosphatase, creatinine, and 17-ketosteroid excretion were determined in a group of children between the ages of 2 and 18 years and in a small group of adults.

For purposes of comparison, the enzyme excretion is best expressed as units per minute but when the possibility of errors in timing the urine sample exists, the results may be expressed as units per mg. of creatinine. The excretion of acid phosphatase varies from hour to hour and day to day. After the age of 12 years males excrete more acid phosphatase than females until at maturity males excrete the enzyme at 3.5 times the rate of females.

There is a general relationship between the excretion of ketosteroids and acid phosphatase in males but the prostate of different individuals varies considerably in the extent to which acid phosphatase is stimulated by a given level of circulating androgen, as reflected by urinary 17-ketosteroid measurements.

Acknowledgment

The authors greatly appreciate the technical assistance of Elizabeth Snook, Mary Davis, Eleanor Clark and Werner Jacobsen. Urine samples from the pre-school age children were collected under the supervision of Dr. Virginia Nelson.

REFERENCES

1. Huggins, C.: The prostatic secretion, *Harvey Lect.* **42**: 148, 1946–47.
2. Huggins, C., and Clark, P. J.: Quantitative studies of prostatic secretions. II. The effect of castration and of estrogen injection on the normal and on the hyperplastic prostate glands of dogs, *J. Exper. Med.* **72**: 747, 1940.
3. Huggins, C.: The physiology of the prostate gland, *Physiol. Rev.* **25**: 281, 1945.
4. Scott, W. W., and Huggins, C.: The acid phosphatase activity of human urine, an index of prostatic secretion, *Endocrinology* **30**: 107, 1942.
5. Hansen, P. F.: Ny Metode til Verdering of Testes interne Sekretions hos manden udfra Prostatasekretets Inhold of "sur" Fosfatase, *Bibliot. f. laeger* **137**: 333, 1945.

6. WOLBERGS, H.: Über Harnphosphatase (die Ausscheidung beim Menschen), *Ztschr. f. Physiol. Chem.* **238:** 23, 1936.
7. CLARK, L. C., and THOMPSON, H.: Determination of creatine and creatinine in urine, *Analyt. Chem.* **21:** 1218, 1949.
8. HUGGINS, C., and TALALAY, P.: Sodium phenolphthalein phosphate as a substrate for phosphatase tests, *J. Biol. Chem.* **159:** 399, 1945.
9. TALBOT, N. B.; BUTLER, A. M.; MACLACHLAN, E. A., and JONES, R. N.: Definition and elimination of certain errors in the hydrolysis, extraction, and spectrochemical assay of α- and β-neutral urinary 17-ketosteroids, *J. Biol. Chem.* **136:** 365, 1940.
10. HERSHBERG, E. B., and WOLFE, J. K.: A rapid extractor for urinary steroids, *J. Biol. Chem.* **133:** 667, 1940.
11. HOLTORFF, A. F., and KOCH, F. C.: The colorimetric estimation of 17-ketosteroids and their application to urine extracts, *J. Biol. Chem.* **135:** 377, 1940.
12. PINCUS, G., and PEARLMAN, W. H.: Fractionation of neutral urinary steroids, *Endocrinology* **29:** 413, 1941.
13. FISHER, R. A.: Statistical Methods for Research Workers, ed. 11, New York, Hafner Publishing Co., 1950, p. 175.
14. CLARK, L. C., and TREICHLER, P.: Psychic stimulation of prostatic secretion, *Psychosom. Med.* **12:** 261, 1950.

A CORRELATION BETWEEN THE SERUM PROTEIN-BOUND IODINE AND THE RADIOIODINE CONVERSION RATIO IN VARIOUS THYROID STATES*

G. E. SHELINE, M.D.,† M. C. MOORE, A. KAPPAS, M.D. AND D. E. CLARK, M.D.

From the Department of Surgery of the University of Chicago, Chicago, Illinois

RECENTLY several tests using radioactive iodine as an indicator of the state of thyroid function have been developed. One of these utilizes a determination of the total plasma radioactive iodine and the protein-bound radioiodine twenty-four hours after the oral administration of an aqueous inorganic I^{131} solution (1, 2). A ratio between these two values had been found to be a useful clinical aid. It is termed the conversion ratio and has been thought to be more or less directly related to the rate at which the thyroid gland utilizes the circulating inorganic iodine of the plasma to form plasma protein-bound iodine. Regardless of how direct the relation may be, it is not a simple one. For example, the amount of inorganic iodine in the body is a factor and patients having received iodine solutions, such as Lugol's, may have a low conversion ratio. Other drugs which influence the activity of the thyroid gland will also exert an effect on this ratio.

The serum or plasma protein-bound iodine as determined chemically has been used successfully to differentiate various thyroid states and apparently correlates well with thyroid activity. In an effort to evaluate the conversion ratio, in terms other than clinical impression and basal metabolic rates, both the conversion ratio and the serum protein-bound iodine have been determined in a series of patients. The series includes most levels of thyroid activity and is not restricted to those subjects who fall distinctly into one classification, such as hyperthyroidism or euthyroidism.

METHOD

The patients used for this investigation were chiefly from the group re-

Received for publication June 16, 1950.

* This work was aided in part by the Charles H. and Mary F. S. Worcester Memorial Fund; by an institutional grant from the American Cancer Society; and by a grant from the U. S. Public Health Service (National Cancer Institute).

The radioactive iodine used in this investigation was supplied by the Oak Ridge National Laboratories, Oak Ridge, Tennessee, and obtained under allocation from the United States Atomic Energy Commission.

† Atomic Energy Commission Postdoctoral Research Fellow in the Medical Sciences of the National Research Council.

TABLE 1. THE SERUM PROTEIN-BOUND IODINE AND THE CONVERSION RATIO IN INDIVIDUALS WITH VARIOUS THYROID STATES

No.	Name	Age	Sex	Clinical diagnosis	P.B.I. μg. %	C.R. %
1.	M.C.	44	F	Euthyroidism	3.8	8
2.	D.D.	3	M	"	3.9	16
3.	M.G.	36	F	"	4.9	3
4.	C.H.	50	F	"	3.4	31
5.	C.H.	48	F	"	4.3	18
6.	J.J.	45	M	"	6.4	7
7.	W.K.	60	M	"	4.5	4
8.	L.K.	64	F	"	2.7	9
9.	M.K.	54	F	"	2.8	16
10.	P.K.	54	F	"	5.1	12
11.	J.L.	29	F	"	3.2	23
12.	L.M.	31	F	"	3.7	32
13.	G.M.	19	M	"	3.0	8
14.	L.R.	30	F	"	3.7	20
15.	F.T.	31	M	"	3.4	4
16.	L.T.	59	M	"	3.9	17
17.	F.W.	63	F	"	4.7	14
18.	C.W.	10	F	"	3.0	23
19.	E.Z.	54	M	"	5.7	8
20.	M.Z.	28	F	"	3.2	34
21.	R.F.	48	M	"	6.0	7
22.	M.P.	2	F	"	4.2	12
23.	C.B.	58	F	Hyperthyroidism, mild	7.0	38
24.	A.B.	62	F	"	10.6	33
25.	H.C.	40	F	"	7.3	82
26.	I.G.	47	F	"	8.1	35
27.	J.B.	36	F	Hyperthyroidism	10.0	81
28.	E.C.	22	F	"	12.5	94
29.	M.E.	41	F	"	8.2	85
30.	J.F.	28	F	"	10.4	57
31.	L.F.	27	F	"	6.8	87
32.	S.G.	46	F	"	11.7	63
33.	A.K.	57	F	"	7.9	80
34.	J.K.	37	F	"	14.0	90
35.	A.L.	59	F	"	12.5	97
36.	A.N.	58	F	"	10.4	45
37.	F.R.	49	F	"	12.1	68
38.	M.S.	28	F	"	13.0	91
39.	P.S.	39	M	"	8.1	40
40.	H.S.	15	F	"	11.8	94
41.	Y.J.	46	F	"	8.8	100
42.	C.A.	1	F	Cretinism	0.5	25
43.	E.C.	38	M	Hypothyroidism	0.8	14
44.	B.L.	1	M	Cretinism	0.8	7
45.	E.Z.	50	F	Myxedema	1.4	4

ferred to this clinic for evaluation of their thyroid status and for therapy if deemed indicated. The use of these cases obviated the necessity of making I^{131} studies in individuals who would not otherwise have received radioactive iodine. In addition to the history and physical examination, routine blood and urine analyses were made. Usually basal metabolic rates were determined. The clinical impressions are recorded in Table 1.

Blood was drawn for determination of the serum protein-bound iodine. Then a test dose of I^{131}, usually between 0.05 and 0.20 millicuries,[1] was given orally. When the patient was definitely hyperthyroid and decision had been made to use radioiodine therapy, the test was occasionally carried out with a therapeutic dose (3). Twenty-four hours later blood was drawn for determination of the conversion ratio.

Conversion ratio

Fifteen ml. of blood were placed in a centrifuge tube containing heparin, mixed, and then centrifuged. Two ml. of plasma were pipetted into a small porcelain dish, dried, and the radioactivity determined with a Geiger-Mueller counter. A similar aliquot was pipetted into a 15 ml. centrifuge cone and the proteins precipitated with 10 per cent trichloroacetic acid. The precipitate was washed twice with 1 per cent trichloroacetic acid, dissolved in dilute NaOH, and transferred to a porcelain dish. This was also dried and its radioactivity determined. The details of the method have been recently described (2). The ratio is calculated as defined: $\frac{\text{protein-bound plasma } I^{131}}{\text{total plasma } I^{131}} \times 100$. The values are expressed in per cent.

Protein-bound serum iodine (I^{127})

The method of Chaney (4), using the Sandell-Kolthoff (5) colorimetric determination for iodide, was chosen for protein-bound iodine estimations. The glass distillation apparatus and the combination centrifuge-digestion-distillation flask described by Connor *et al.* (6) were used with certain modifications. The preparation and amounts of reagents used were according to Connor *et al.* (6).

Three ml. of serum were pipetted into the distillation flask. Somogyi reagents were added and the proteins thus precipitated with zinc hydroxide. After centrifugation, the supernatant solution was poured into a small flask and saved for determination of the inorganic iodide. The precipitate was then washed with 20 ml. of water, recentrifuged, and the supernatant discarded. Three ml. of 50 per cent solution of chromic oxide and 25 ml. of a 70 per cent solution of sulfuric acid were added and the protein digested by heating. The solution was cooled, 15 ml. of distilled water were added and the mixture heated to 185° C. This step was repeated once more to insure removal of volatile substances. Thirty ml. of distilled water were added, the flask attached to the glass still, and heated. As soon as the solution reached a steady boil, 5 ml. of a 50 per

[1] Oak Ridge Standard.

cent solution of phosphorous acid were added through a dropping funnel. One ml. of a 1 per cent solution of KOH had previously been placed in the receiving tube. Distillation was continued for ten minutes. The receiving tube was disconnected and the lead-in tube was washed down with distilled water. The distillate was transferred with small washings into a specially selected Evelyn colorimeter tube and diluted to 10 ml. Arsenious acid, hydrochloric acid and ceric sulfate solutions (6) were added and multiple readings made in an Evelyn colorimeter, using a 440 millimicron filter. The readings were made at intervals up to ten minutes, depending on how rapidly the color of the solution faded. The values so obtained were compared with calibration curves previously obtained by a similar treatment of known amounts of KI. The inorganic iodine was also determined colorimetrically.

Essentially four modifications of the apparatus of Connor *et al.* (6) were made. Digestion and distillation were found to proceed more smoothly if the diameter of the flask was increased to 62 mm. This size was chosen because the inside diameter of our 250 cc. centrifuge cups was 63 mm. A trap was placed in the distillation line to prevent the rather troublesome suck-back which frequently occurred. Use of pyrex receiving tubes with standard taper joints made it easier to obtain interchangeable parts from our glass blower. It was found necessary to reduce to about 0.6 mm. the size of the capillary at the end of the tube leading back from the condenser to the distilling flask. The 1 mm. diameter capillary would occasionally allow escape of the first portion of the distillate.

All samples were run in duplicate and the results averaged. In most cases the values differed by less than 10 per cent. If the difference reached 15 per cent, the determinations were repeated. In 2 hyperthyroid patients, four determinations gave values of 14.8, 13.9, 13.1, 14.0 (average 14.0) μg. per cent and 18.0, 18.1, 19.3, 19.3 (average 18.7) μg. per cent, respectively. These results are indicative of the reproducibility obtained. Blank determinations on the reagents yielded values of about 0.02 μg. of iodine.

In order to establish the normal range of serum protein-bound iodine by the technique used in our laboratory, individual determinations were made in duplicate on 50 euthyroid subjects. The results are shown in Figure 1. The values ranged from 2.9 μg. per cent to 5.9 μg. per cent, with an average of 4.2 μg. per cent. In the evaluation of the subsequent studies, values between 2.4 μg. per cent and 6.4 μg. per cent were considered to represent euthyroidism.

RESULTS

The serum protein-bound iodine and the conversion ratio were determined in 22 euthyroid, 19 hyperthyroid and 4 hypothyroid individuals (Table 1). In the euthyroid subjects, the serum protein-bound iodine ranged from 2.7 μg. per cent to 6.4 μg. per cent and the conversion ratios fell between 4 and 34 per cent. The serum protein-bound iodine levels for the hyperthyroid group were 6.8 μg. per cent or higher. The lowest conversion ratio in this group was 33 per cent. The conversion ratios in the hypo-

thyroid patients varied between 4 per cent and 25 per cent and the serum protein-bound iodine ranged from 0.5 μg. per cent to 1.4 μg. per cent. The serum protein-bound iodine level and the conversion ratios for each individual are correlated in Figure 2.

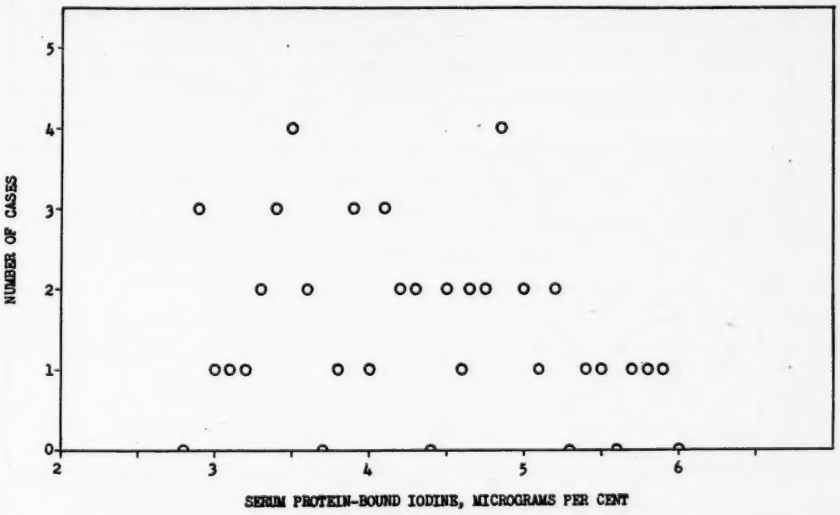

FIG. 1. Showing the frequency distribution of the serum protein-bound iodine levels in 50 euthyroid individuals.

DISCUSSION

The serum protein-bound iodine levels, in patients who were considered clinically to have thyrotoxicosis, were all above the normal range. The euthyroid individuals had levels within the limits established for normal and the 4 hypothyroid patients had values below the normal range. With 3 exceptions the individuals with elevated serum protein-bound iodine levels had conversion ratios of 40 per cent or higher and only 1 of these had a ratio below 35 per cent. In the euthyroid group no conversion ratio exceeded 35 per cent. Figure 2 shows that when either the values for the serum protein-bound iodine or the conversion ratio are considered, the higher values in the euthyroid group nearly equal or only slightly exceed the lower values of the hyperthyroid group. It appears from this group of cases that the conversion ratio is of approximately equal value to the serum protein-bound iodine in the differentiation of individuals with euthyroidism from those with hyperthyroidism.

Within the hyperthyroid group there was no consistent correlation be-

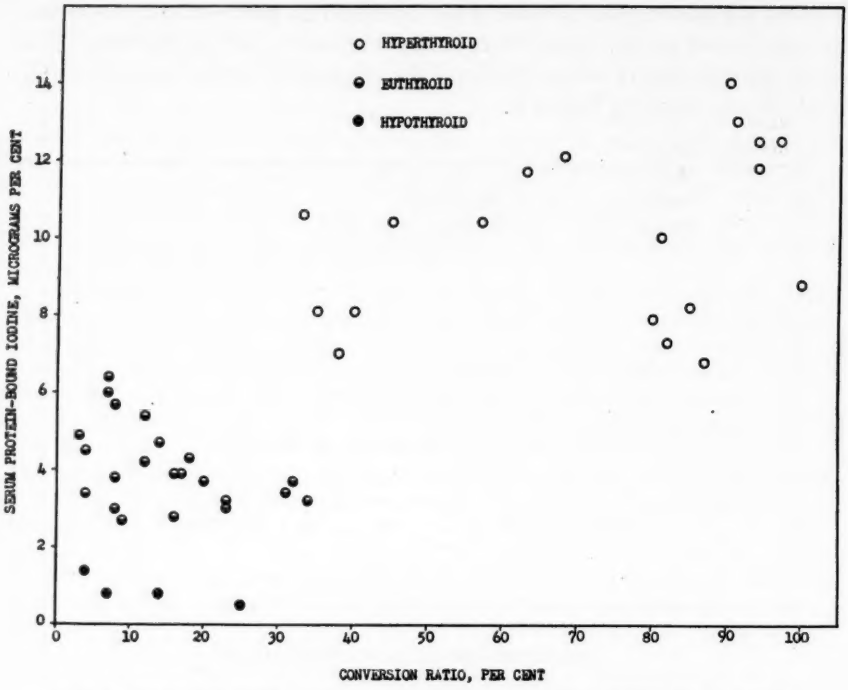

FIG. 2. Showing the correlation between the serum protein-bound iodine and the conversion ratio in patients with various thyroid states.

tween the level of the serum protein-bound iodine or of the conversion ratio and the severity of the thyrotoxicosis.

A considerable number of euthyroid subjects had a conversion ratio of 10 per cent or under. Only 4 cases of hypothyroidism were studied. These had conversion ratios of 4, 7, 14 and 25 per cent. Thus in this limited group the conversion ratios fell within the range found for euthyroidism.

SUMMARY

The conversion ratio of radioiodine is correlated with the protein-bound iodine in the serum of individuals with various thyroid states. These determinations were found to be of approximately equal value in distinguishing between euthyroidism and hyperthyroidism. The conversion ratio did not distinguish 4 cases of hypothyroidism from the euthyroid group, whereas the serum protein-bound iodine did.

The methods for determining the conversion ratio and the serum protein-bound iodine are briefly discussed.

REFERENCES

1. Clark, D. E.; Moe, R. H., and Adams, E. E.: The rate of conversion of administered inorganic radioactive iodine into protein-bound iodine of the plasma as an aid in the evaluation of thyroid function, *Surgery* **26:** 331–340 (Sept.) 1949.
2. Sheline, G. E., and Clark, D. E.: Index of thyroid function: estimation by rate of organic binding of I^{131}, *J. Lab. & Clin. Med.* **36:** 450–455 (Sept.) 1950.
3. Moe, R. H.; Adams, E. E.; Rule, J. H.; Moore, M. C.; Kearns, J. E., and Clark, D. E.: An evaluation of radioactive iodine in the treatment of hyperthyroidism, *J. Clin. Endocrinol.* **10:** 1022–1028 (Sept.) 1950.
4. Chaney, A. L.: Improvements in determination of iodine in blood, *Indust. & Engin. Chem.*, Anal. Ed. **12:** 179–181 (Mar.) 1940.
5. Sandell, E. B., and Kolthoff, I. M.: Chronometric catalytic method for the determination of micro quantities of iodine, *J. Am. Chem. Soc.* **56:** 1426 (June) 1934.
6. Connor, A. C.; Swenson, R. E.; Park, C. W.; Gangloff, E. C.; Lieberman, R., and Curtis, G. M.: The determination of the blood iodine. A useful method for the clinical laboratory, *Surgery* **25:** 510–517 (April) 1949.

ACTION OF ESTROGENS ON RELEASE OF LUTEINIZING HORMONE IN MENOPAUSAL WOMEN*†

JOSEPH W. FUNNELL, M.D., CORINNE KEATY, PH.D. AND ARTHUR A. HELLBAUM, PH.D., M.D.

From the Department of Pharmacology, Oklahoma University School of Medicine, Oklahoma City, Oklahoma

ALTHOUGH estrogens and androgens exert an inhibiting influence on the release of the follicle-stimulating (FSH) factor from the hypophysis, these same substances stimulate the release of the pituitary luteinizing factor (LH) in laboratory animals. As early as 1930, Scaglione (1) and in 1934, Hohlweg (2) demonstrated that ovaries of adult and immature normal rats could be luteinized by administration of estrogens. Hellbaum and Greep (3, 4) reported that castration in both male and female rats is followed by a marked increase in the amount of LH present in the pituitary gland as compared to normal rats. The latter investigators also found that the stored LH could be liberated by estrogens or androgens, so that following the administration of these steroids to castrated animals for from thirty to forty-five days the pituitaries contained only FSH and induced only follicular stimulation in the ovaries of immature test rats.

The purpose of this study was to determine if these findings might also be observed in human subjects. Patients undergoing the menopause, either surgical or physiologic, were selected for this investigation because they present a definite symptom complex, and excrete primarily follicle-stimulating hormone in the urine. It was postulated that in the absence of estrogenic stimulation in untreated menopausal patients the LH remains stored in the pituitary gland and only FSH is liberated, resembling the condition in castrated laboratory animals. Therefore the administration of estrogens should stimulate the release of the stored LH, which in turn would be evidenced by increased excretion of LH in the urine.

METHODS AND MATERIALS

Seven patients, all experiencing severe menopausal symptoms, were studied. These patients were followed over a period of several months,

Received for publication June 23, 1950.

* Read at the Annual Meeting of the Association for the Study of Internal Secretions, Atlantic City, June 4, 1949.

† This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

during which time they received a number of courses of treatment with relatively large doses of estradiol benzoate[1] and ethinyl estradiol.[1] Between successive courses of treatment the patients were given rest periods of such duration that their symptoms returned to pretreatment severity. Urinary gonadotropins were assayed before, during, and after treatment. Ninety-nine gonadotropin assays were performed on 49 urine specimens from these patients during the time they were not receiving estrogens. In some cases these assays were conducted before treatment was instituted, and at other times following withdrawal of treatment. Forty-two assays were carried out on 18 urine specimens of these same patients during the time they were receiving courses of treatment with estradiol benzoate in dosages of 1.66 to 3.32 mg. daily. Eighteen urinary gonadotropin assays were performed on 11 urine specimens while the patients were receiving ethinyl estradiol in dosages of 0.1 to 0.2 mg. daily.

The urinary hypophyseal gonadotropins were concentrated from 24-hour and 12-hour overnight urine specimens by the ultrafiltration (5) or the tannic acid precipitation (6) method. The gonadotropic concentrates were tested on normal and hypophysectomized immature female rats. Ovarian weights and the presence or absence of luteinization were used as end points. Each specimen was assayed as follows: The dry residue from the 24-hour urine specimen was divided into 4 equal parts by weight and 2 or 3 of these portions were assayed on normal rats. If only follicular stimulation was observed, it was concluded that only FSH was being excreted in the urine, for it has been shown that even small amounts of LH in the presence of FSH will cause luteinization. If definite luteinization or any evidence of luteinization such as cloudy follicles or thickened theca interna was observed in the ovaries of the normal rats, the remaining aliquot was assayed on hypophysectomized rats in order to rule out the participation of the endogenous pituitary activity. In those instances in which luteinization was observed in both normal and hypophysectomized rats, it was concluded that LH was being excreted in the urine.

RESULTS

Gonadotropin assays carried out on the 49 urine specimens collected when the patients were not receiving estrogens, revealed that the ovaries of 74 of the test animals showed only follicular stimulation, whereas the ovaries of the remaining 25 animals, showed varying degrees of luteinization. The observed luteinization may have been due in part to activity of the test animal's own pituitary, or to the actual presence of LH in the urine. It is felt that in most instances it was due to endogenous secretion

[1] Kindly furnished by the Schering Corporation, Bloomfield, New Jersey.

of LH, for luteinization was observed in only 1 of the 22 hypophysectomized animals used in the assays of the urine collected during the time the patients were not receiving estrogens.

In contrast, marked luteinization was observed in the ovaries of 54 of the 60 test animals used to assay the 29 urine specimens collected during the time these same patients were receiving the relatively large doses of estrogens. Follicular development alone was observed in the ovaries of only 6 of these test animals, compared to 74 of the 99 used to assay the urine specimens collected when the patients were not receiving estrogens.

TABLE 1. SUMMARY OF RESULTS OF ESTROGEN THERAPY IN 7 MENOPAUSAL PATIENTS

Treatment	No. of specimens	Assays	Response of test ovaries		Response of patient
			F*	L†	
None	49	99	74	25	Hot flushes (6–23 daily)
Estradiol benzoate 1.66–3.32 mg.	29	60	6	54	Asymptomatic
Ethinyl estradiol 0.1–0.2 mg.					

* F—Follicular stimulation.
† L—Luteinization.

The ovarian weights of the test animals of both groups fell within the same range. Thus it would seem that during the time these patients were receiving estrogens, the luteinizing factor was released from their pituitaries in sufficiently increased amounts to be constantly demonstrable in the urine; whereas, during the time when these same individuals did not receive estrogens, LH was not released, at least not in sufficient quantities to be demonstrated in the vast majority of the test animals, by the method of concentration and assay employed.

DISCUSSION

The patients in this investigation were chosen with care and only those individuals whose symptoms were not altered by carefully used placebos were included in the study. Since the hot flush (not the temporary hot flash) is the most consistent and characteristic symptom of the menopause, the number, duration, and severity of hot flushes was used as the chief

index of the patients' symptomatic response to treatment. Only those patients were studied who had been experiencing hot flushes of such severity before treatment that they awakened at night. It was felt that these were more than psychic manifestations, and represented genuine vasomotor instability.

During the intervals in which the subjects were not receiving estrogens, menopausal symptoms were severe, and various patients were experiencing from 6 to 23 hot flushes during a 24-hour period. All the patients became asymptomatic during treatment with relatively large doses of estrogens. It was observed that coincidentally with symptomatic relief, which in most instances occurred on the second day of treatment, LH was excreted in the urine in increased amounts. It was also of interest to note that with small quantities of estrogens the patients were afforded symptomatic relief, but that LH was not demonstrated in the urine by the method of concentration and assay. It may be that the method is not sufficiently sensitive to detect minute amounts of LH. A limited number of tests indicate that LH is not released as readily by stilbestrol and its derivatives as by the cyclopentenophenanthrene type of steroids.

SUMMARY

A total of 159 gonadotropin assays were carried out on urine specimens of 7 menopausal patients. During the time that these patients were not receiving estrogens, their menopausal symptoms were severe and they excreted primarily follicle-stimulating hormone (FSH) in the urine; whereas during treatment with relatively large amounts of estradiol benzoate or ethinyl estradiol they experienced complete symptomatic relief, which was coincidental with the demonstrable excretion of increased amounts of LH in the urine.

These findings indicate that in the absence of sufficient estrogens to stimulate the release of LH, as is the case in menopausal women, LH remains in the pituitary. On the other hand, the administration of estrogens in sufficient quantities stimulates the release of LH from the pituitary so that LH is excreted in demonstrable amounts in the urine.

The storage or liberation of the separate pituitary gonadotropic hormones (FSH and LH) are independently influenced by estrogens, and the intracellular synthesis of these factors is a separate process from that involving the liberation of these factors from the gland.

REFERENCES

1. Scaglione, S.: Ormone follicolare e ovaio, *Riv. ital. di ginec.* **1**: 463–471 (Nov.) 1930.
2. Hohlweg, W.: Veränderungen des Hypophysenvorderlappens und des Ovariums nach Behandlung mit grossen dosen von Follikelhormon, *Klin. Wchnschr.* **13**: 92–95 (Jan. 20) 1934.

3. HELLBAUM, A. A., and GREEP, R. O.: Qualitative changes induced in gonadotropic complex of pituitary by testosterone propionate, *Endocrinology* **32**: 33–40 (Jan.) 1943.
4. HELLBAUM, A. A., and GREEP, R. O.: Action of estrogen on release of hypophyseal luteinizing hormone, *Proc. Soc. Exper. Biol. & Med.* **63**: 53–56 (Oct.) 1946.
5. GORBMAN, A.: Ultrafiltration of urine for collection and biological assay of excreted hypophyseal hormones, *Endocrinology* **37**: 177–190 (Sept.) 1945.
6. HELLBAUM, A. A.; FEVOLD, H. L., and HISAW, F. L.: Method for concentrating the gonadotropic activity in pregnancy urine, *Proc. Soc. Exper. Biol. & Med.* **32**: 1566–1567 (June) 1935.

THE GLYCOGEN-ESTROGEN RELATIONSHIP IN THE VAGINAL TRACT

W. BURTON AYRE, M.D.

From the Cytological Laboratories, Donner Building for Medical Research, McGill University, Montreal, Canada

A DIRECT relationship between estrogenic activity and the concentration of glycogen in the vaginal epithelium has been stressed by a number of investigators (1, 2, 3, 4, 5). There has been evidence to show that the glycogen content of the vaginal fluid is similarly influenced (5, 6, 7, 8, 9). Accordingly, the percentage of glycogen-containing cells in the epithelial smear (the glycogen-index) has been applied as an index of estrogenic activity (8, 9). It was recently demonstrated (10), however, that the glycogen index is not reliable when hyperestrinism is induced. With further study it has been observed that changes in the vaginal fluid upon estrogen withdrawal following hyperestrinism are also unusual. These further observations are described in the following report. Since the characteristics of the vaginal smear under these conditions are not in conformity with the glycogen-estrogen relationship as previously described, a modified concept of this relationship is suggested.

The previously reported study (10) set forth data on 6 subjects with small anaplastic lesions of the cervical epithelium, who were treated with estrogens. Vaginal smears were taken according to Papanicolaou's technique (11) and stained for glycogen with Best's carmine (12). When hyperestrinism was established the vaginal smear was found to be glycopenic in character. Further study has shown that upon the withdrawal of estrogen the glycogen content of the vaginal fluid remains unchanged for a variable period. It then increases and glycogen is found in the smear in appreciable concentrations. The reappearance of glycogen in large amounts was particularly striking in 2 cases in which the effect of ovarian activity was excluded. A paradoxically high vaginal fluid glycogen content persisted, in these cases, over a period of several months of apparent estrogen deprivation. Subsequently, the amounts present gradually decreased and glycopenia developed, coinciding with the development of epithelial atrophy.

A brief protocol of each of these 2 cases follows:

Case I. Mrs. L. S., aged 33, para 3. Subsequent to a two-month course of stilbestrol therapy, carcinoma of the cervix developed in this patient. Therapy was discontinued in February, 1949. Total hysterectomy and bilateral ovariectomy were performed in

Received for publication June 19, 1950.

April, 1949. Details of therapy and studies undertaken to the time of operation have been reported previously (13). Eighteen vaginal smears were obtained during the interval between cessation of therapy and operation. Most were glycopenic in character (Fig. 1). The predominant cell type present was the superficial squamous cell (Fig. 2).

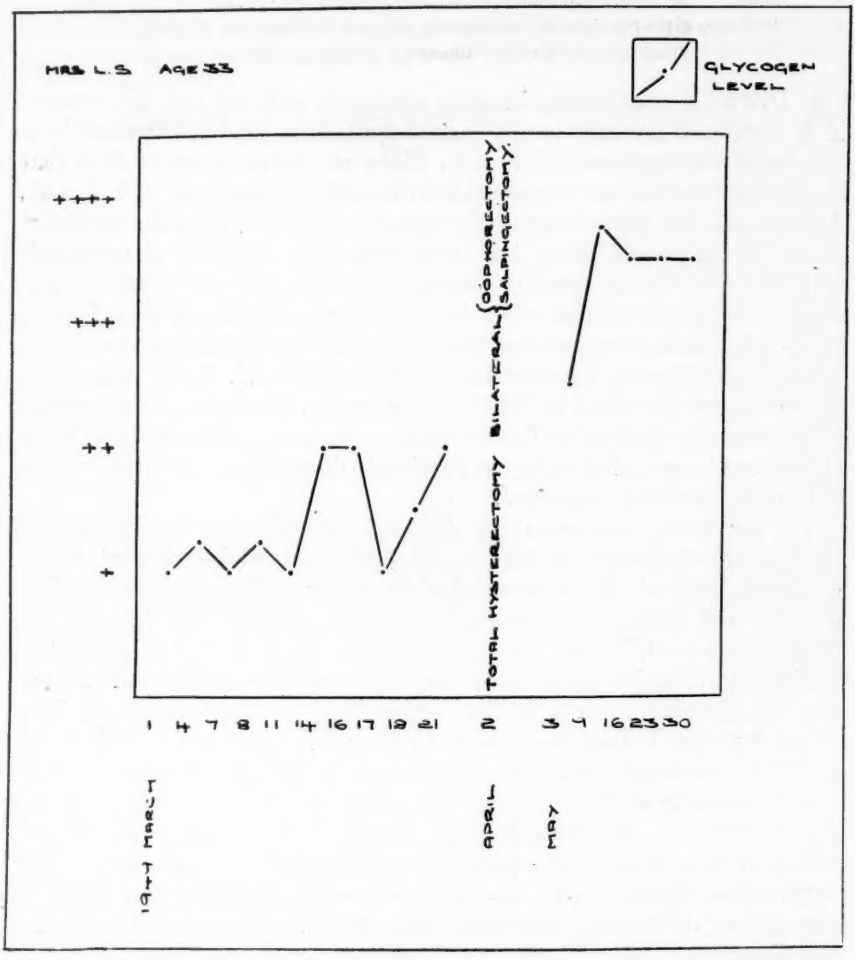

FIG. 1. Graphic illustration of changes in the glycogen content of the vaginal fluid following estrogen withdrawal and bilateral ovariectomy in a subject with previously induced hyperestrinism. The administration of stilbestrol was discontinued on February 15, 1949. The vaginal smear was glycopenic following therapy, as indicated. In May 1949, after bilateral ovariectomy, repeated smears showed a high concentration of glycogen.

Glycogen became more conspicuous in the smear a week prior to operation, with the appearance of intermediate cells. An interval of one month, during which no smears were obtained, followed operation. In the following month, 5 smears were taken at regular intervals, each of which contained a relatively high concentration of glycogen (Fig. 1). The predominant cell type was from the intermediate layer (Fig. 3). Throughout the subsequent nine months 6 smears obtained at intervals of from one to two months showed declining glycogen indices. A final smear obtained February 17, 1950 contained 25 per cent of glycogen-positive cells. Basal cells were present in the smear at this

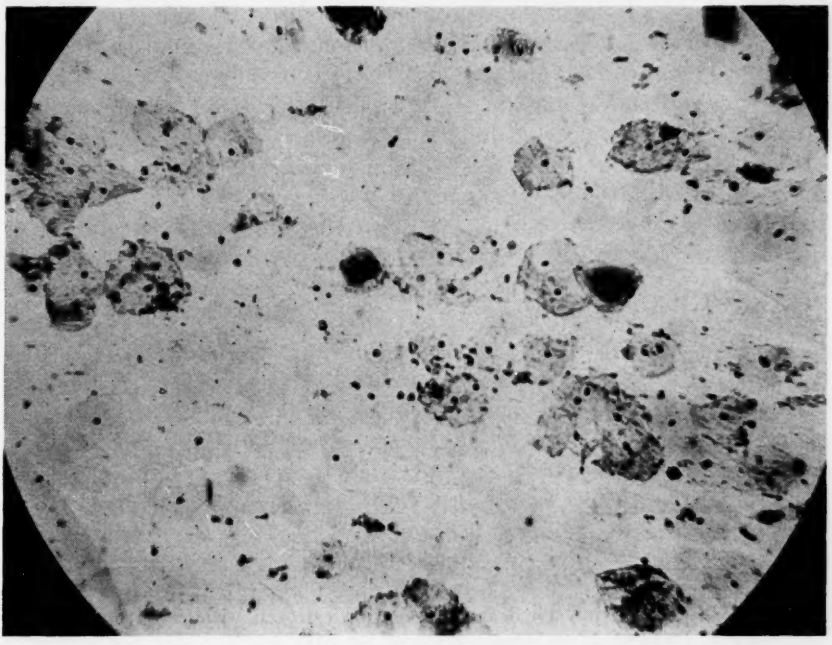

FIG. 2. Glycopenic vaginal smear following induced hyperestrinism. (Subject L. S., smear prepared on March 17. The glycogen content is indicated in Figure 1 as +). Note the flat angular squamous cells of which the smear is composed. These are apparently derived from a hyperkeratotic epithelium. Only an occasional cell contains glycogen.

time, indicating that advanced atrophy had developed in some areas of the vaginal tract.

Case II. Mrs. E. M., aged 36, para 1, had had a subtotal hysterectomy and a bilateral ovariectomy at the age of 19. Significant details on therapy and alterations in vaginal smear glycogen following estrogen administration have been described previously (10). After a period of approximately four months, during which a total of 3,900 mg. of stilbestrol dipropionate was administered, the estrogen was withdrawn on April 25, 1949. For a follow-up study, 71 smears were taken at fairly regular intervals over a period of

twelve months. Until early July, 1949, the smear was composed of superficial cornified cells and remained glycopenic in character. Then glycogen began to be more conspicuous, again coinciding with the appearance of intermediate cells in the smear. Throughout July and August, 22 smears were obtained, all of which were composed predominantly of intermediate cells with a relatively high content of glycogen. During the eight subsequent months, a series of 49 smears was obtained. This series reflected a gradual decline in the glycogen content of the vaginal fluid and in the final smear, obtained April 6, 1950, a glycogen index of 10 per cent was found.

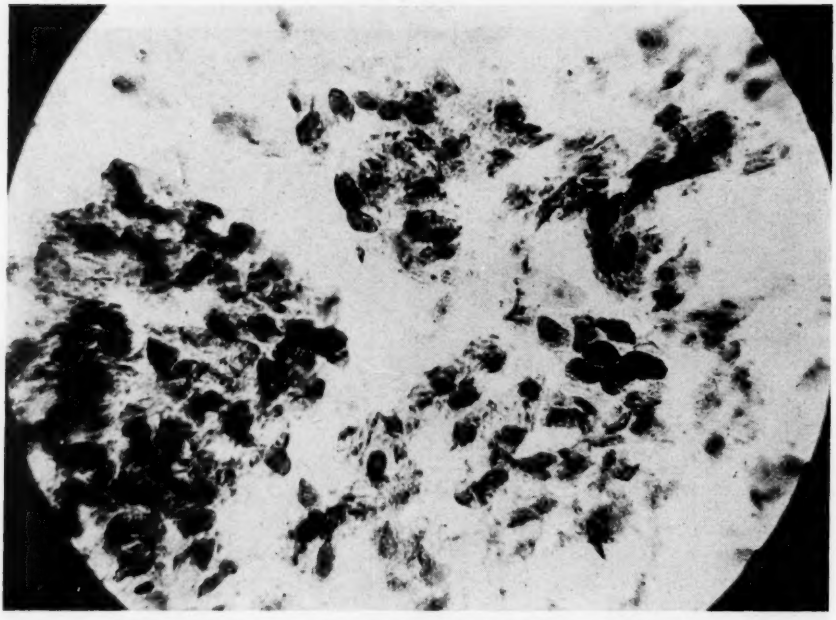

FIG. 3. Glycogen-rich vaginal smear following estrogen withdrawal and bilateral ovariectomy. (Subject L. S., smear prepared on May 16. The glycogen content is evaluated in Figure 1 as ++++.) The smear is now composed of rounded intermediate cells most of which stain heavily with Best's carmine, indicating the presence of a high concentration of glycogen.

In each of these special cases the withdrawal of administered estrogen was followed by an increase in the glycogen content of the smear. This increase did not occur however until two or three months had elapsed and was then associated with a change in the predominant cell type present. Superficial cornified cells, few of which contained glycogen, were replaced by glycogen-rich cells from the intermediate stratum of the epithelium. While this cell type was predominant—a period of a further two to three months—the smear contained appreciable quantities of glycogen. The amount present in the intermediate cell tended to diminish toward the

end of this period, presumably due to ageing. There were still however, high percentages of glycogen-positive cells in the smears approximately five months after estrogen withdrawal. Since the effects of ovarian activity were also excluded, it becomes a problem to define the exact relation between this series of changes in vaginal smear glycogen and estrogenic activity.

If the glycogen content of the vaginal fluid were directly related to estrogenic activity one would not expect a rise in its concentration and its maintenance at a relatively high level over a prolonged period, following estrogen withdrawal and following castration, as in these 2 cases. In the series of changes reflected by the vaginal smear the character of the epithelium, per se, appeared to play a more prominent role than the direct effect of estrogenic activity. Initially, following estrogen withdrawal, the smear remained glycopenic for approximately two months in each case. During this period, the cells present were flake-like with small pyknotic nuclei, and were apparently derived from the superficial layer made hyperkeratotic by prolonged estrogen administration. When this layer was desquamated, more rounded, intermediate cells containing glycogen were found. This cell type predominated in the glycogen-rich smear in each case. As the intermediate layer, in turn, was desquamated basal cells containing no glycogen appeared and the smear became glycopenic.

Hyperkeratotic areas of the vaginal epithelium do not contain glycogen in the superficial layers,—a fact repeatedly demonstrated by Schiller (14) who has found no glycogen in such areas when painted with iodine. The subsurface layers in these areas do however contain glycogen. In one case in the group studied, a biopsy specimen of the vaginal epithelium showed glycogen to be present in normal concentration in the intermediate layer, whereas the smear was glycopenic in character. The rich concentration in the intermediate layer has been repeatedly stressed; during pregnancy (4) and at various times during the menstrual cycle, of all portions of the epithelium, this stratum (5, 15) contains the highest concentration of glycogen. In contrast, glycogen is usually absent from the basal layer.

The sequence of changes observed with induced hyperestrinism and withdrawal therefore suggest that this histochemical characteristic of the epithelial cell, as examined in the smear, depends primarily upon the layer of the epithelium from which it is derived. While the hyperkeratotic layer is intact the smear is glycopenic; when the glycogen-rich intermediate stratum is exposed the smear has a high glycogen content; when the basal layer, in turn, is exposed the smear is again glycopenic. Epithelial topography or architecture, rather than direct estrogenic action, appears to have been the essential factor which determined the glycogen content of the vaginal smear in these cases.

The possible significance of epithelial structure, per se, in relation to glycogen accumulation in the tissue is also emphasized by this study. Its concentration in the intermediate layer continued at appreciable levels, according to the characteristics of the smear, for at least four months after estrogen withdrawal and castration, and only disappeared with beginning epithelial atrophy and the disappearance of this stratum. It appears, therefore, that while intact, the various layers of the epithelium contain glycogen in characteristic concentrations despite changes in estrogenic activity. In this regard the vaginal epithelium resembles such topographically similar simple stratified membranes as the buccal and esophageal epithelia. The concentration of glycogen in each of the latter is not usually as great as in the vaginal epithelium but this may be due to such factors as environmental differences, rapidity of growth, and intensity of cell activity. It is notable that in each of these simple stratified epithelia glycogen is most conspicuous in the intermediate layers where the cell is far removed from its blood supply. A tendency for glycogen to accumulate in areas of relative avascularity has been frequently observed. Numerous examples are cited by Dempsey and Wislocki (16) who have suggested that this factor may influence the accumulation of glycogen in the endometrium. From their original investigations, and the examples cited, they have deduced that glycogen deposition is generally associated with anaerobic conditions. The reason for this is not known but it is conceivably related to the failure of aerobic mechanisms, with developing ischemia, and the capacity of glycogen synthesizing enzymes to remain active in the anoxic environment. It is possible, therefore, that the accumulation of glycogen in simple stratified epithelia, including the vaginal epithelium, is mediated by some factor in the metabolism of the epithelial cell which is brought into play at one point of the progressive devitalization it undergoes in its life cycle. This hypothesis suggests that estrogens act primarily as growth stimulants and cell differentiators, producing a multilayered epithelium, and that certain cells of the epithelium thus formed accumulate glycogen as a consequence of their intrinsic metabolic activity. Although the structure of the epithelium is influenced by estrogens, this intrinsic metabolic process may, in itself, be unaffected by the prevalent degree of estrogenic activity.

On the other hand, the suggestion that estrogens are directly concerned in the metabolism of epithelial glycogen is implicit in many of the reports. The direct action of estrogens in mobilizing glycogen in the vaginal tract has been repeatedly demonstrated (3, 5, 6, 7, 8). Its concentration in the epithelium, within certain limits, is definitely related to the degree of estrogenic activity. Following estrogen withdrawal, the amount present decreases. Van Dyke and Ch'en (3) following a series of experiments concluded that no method short of bilateral ovariectomy, which removes the

estrogenic stimulus, will reduce the glycogen content of the vaginal epithelium. Salmon *et al.* (17) have shown that this may also be accomplished by antagonizing estrogenic action with administered testosterone. Rakoff *et al.* (5) have stated, on this basis, that androgens appear to be inhibitory to glyconeogenesis.

It is possible, however, to interpret these established facts in a somewhat different manner. Estrogenic activity may directly influence the concentration of glycogen present since it increases the height of the epithelium and the number of cells in the intermediate stratum, which presumably possesses the inherent capacity to accumulate it. Conversely, androgens, which reduce the glycogen content of the epithelium, first induce epithelial atrophy and therefore prevent the development of this type of cell. The action of both estrogens and androgens upon vaginal glycogen may therefore be explained primarily by their influence upon growth activity and cell differentiation rather than by stimulation or inhibition of glyconeogenesis or glycogenesis.

SUMMARY

It has been previously demonstrated that the vaginal smear accompanying induced hyperestrinism is usually glycopenic in character. In this report, changes which occur upon estrogen withdrawal following induced hyperestrinism, are described. Detailed studies on 2 patients in whom it was possible to exclude the effects of ovarian activity are presented. It was found that, following a variable interval in which the aglycocytic hyperkeratotic layer produced by prolonged estrogen administration is sloughed off, glycogen reappears in the vaginal fluid in relatively high concentrations. Its reappearance in appreciable amounts only follows however upon the exposure of the glycogen-rich intermediate layer of the epithelium. As this layer, in turn, is desquamated, basal cells containing no glycogen appear and the smear is again glycopenic. It is concluded that the concentration of glycogen in the vaginal fluid is determined primarily by the topography or architecture of the intact epithelium.

The results suggest, further, that the various *intact* layers of the vaginal epithelium contain glycogen in characteristic concentrations independent of estrogenic activity. It may be inferred, therefore, that the structural characteristics of the epithelium primarily determine its glycogen content. Accordingly, the influence of estrogens and androgens upon the amounts present may result from their action upon epithelial growth and differentiation. Glycogenesis, at this site, may be essentially an expression of the inherent properties of the differentiated epithelial cell—an intrinsic metabolic process which is only indirectly influenced by estrogenic activity.

Acknowledgments

This work was supported by a grant from the Public Health Foundation for Cancer and Blood Pressure Research, Inc., Stamford, Connecticut. The technical assistance of Miss Pamela Mapleson, and Miss Barbara Millar is gratefully acknowledged. Endocrine preparations used were generously supplied by Charles E. Frosst and Company, Montreal, and Ciba Pharmaceutical Products, Montreal. The photomicrographs were prepared by Mr. E. A. Stanger and Mr. F. Hopkins.

REFERENCES

1. Miura, H.: *Mitt. a. d. med. Akad. zu Kioto* **2:** No. 1, 1928. Cited by Davis and Pearl (4).
2. Cruickshank, R., and Sharman, A.: The biology of the vagina in the human subject, *J. Obst. & Gynaec. Brit. Emp.* **41:** 190–207, 1934.
3. Van Dyke, H. B., and Ch'en, G.: Observations on the biochemistry of the genital tract of the female macaque particularly during the menstrual cycle, *Am. J. Anat.* **58:** 473–492, 1936.
4. Davis, M. E., and Pearl, S. A.: Biology of the human vagina in pregnancy, *Am. J. Obst. & Gynec.* **35:** 77–100, 1938.
5. Rakoff, A. E.; Feo, L. G., and Goldstein, L.: The biologic characteristics of the normal vagina, *Am. J. Obst. & Gynec.* **47:** 467–494, 1944.
6. Robertson, D. C.; Maddux, W. P., and Allen, E.: Ovarian hormone effects in ovariectomized monkeys, *Endocrinology* **14:** 77–88, 1930.
7. Herrenberger, K., and Horstmann, F. H.: Der Glykogengehalt des kindlichen vaginal Epithels und seine Veränderung nach Follikelhormongaben, *Arch. f. Gynäk.* **108:** 451–458, 1939.
8. Mack, H. C., and Ale, T.: Appraisal of estrogenic activity by the vaginal glycogen index; a comparison of oral and parenteral estrone, *J. Clin. Endocrinol.* **2:** 361–364, 1942.
9. Mack, H. C.: The glycogen index in the menopause, *Am. J. Obst. & Gynec.* **45:** 402–418, 1943.
10. Ayre, W. B., and Ayre, J. E.: Vaginal smear glycogen: limitations as an index of estrogenic activity, *J. Clin. Endocrinol.* **9:** 1359–1361, 1949.
11. Papanicolaou, G. N.: The sexual cycle in the human female as revealed by vaginal smears, *Am. J. Anat.* **52** (Suppl.): 519–637, 1933.
12. Mapleson, P.: A modified Best's carmine technique for staining glycogen in vaginal smears, *Canad. Tech. J.* **2:** 166–167, 1949.
13. Ayre, J. E., and Ayre, W. B.: Progression from "precancer" stage to early carcinoma of cervix within one year, *Am. J. Clin. Path.* **19:** 770–778, 1949.
14. Schiller, W.: Leucoplakia, leucokeratosis, and carcinoma of the cervix, *Am. J. Obst. & Gynec.* **35:** 17–38, 1938.
15. Papanicolaou, G. N.; Traut, H. F., and Marchetti, A.: The Epithelium of Women's Reproductive Organs. New York, The Commonwealth Fund, 1948.
16. Dempsey, E. W., and Wislocki, G. B.: Observations on some histochemical reactions in the human placenta, with special reference to the significance of the lipoids, glycogen and iron, *Endocrinology* **35:** 409–429, 1944.
17. Salmon, U. J.; Walter, R. I., and Geist, S. H.: Effect of testosterone propionate on glycogen content of human vaginal smears, *Proc. Soc. Exper. Biol. & Med.* **39:** 467–470. 1938.

Letter to the Editor

IODOTHIOURACIL IN THE TREATMENT OF HYPERTHYROIDISM

To the Editor:

From September 1948 to date, over 110 of our patients have received 5-iodo-2-thiouracil (sodium salt) as medical treatment for hyperthyroidism or as preparation for thyroidectomy. This new antithyroid compound has the apparent advantage of being less toxic and less goitrogenic than the other thiourea derivatives in use up to this time. It has the distinct advantage of producing changes in the thyroid gland that make surgical resection of this organ easier to perform. This new drug can be given up to the day of operation without any supplemental treatment with iodine.

The optimum therapeutic dose of iodothiouracil appears to be between 300 mg. and 400 mg. daily. Control of the hyperthyroidism has been difficult with this new compound in patients suffering from toxic diffuse goiter associated with marked emotional disturbances. Intercurrent infections have also caused trouble in maintaining control of hyperthyroidism. These disadvantages require further investigation.

Clinical studies comparing iodothiouracil and propylthiouracil given with or followed by Lugol's solution are being completed for a more detailed report.

John C. McClintock, M.D.
John J. A. Lyons, M.D.

149½ Washington Avenue,
Albany 6, N. Y.

The Association for the Study of Internal Secretions

THE 1951 ANNUAL MEETING

The Thirty-third Annual Meeting of the Association for the Study of Internal Secretions will be held at the Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey. The Scientific Sessions have been extended to three days, Thursday, Friday, and Saturday, June 7-8-9.

The Local Chairman of Arrangements is Dr. Matthew Molitch.

Due to the advance in the date of our meeting, Chalfonte-Haddon Hall Hotel will be unable to accommodate all of our members by reason of a previous commitment. The Colton Manor, on Pennsylvania Avenue near Haddon Hall, is an excellent hotel, which has been recently refurnished, and is holding one hundred rooms for our members on the "first come, first served" basis. The Hotel Strand, at Pennsylvania Avenue and the Boardwalk, has also promised to supply a limited number of rooms. It is important that reservations be made immediately if you are to avoid disappointment.

All Scientific Sessions will be held in the Chalfonte-Haddon Hall Hotel. The rooms, in which the session will be held, will be announced in the program and on the hotel bulletin board. The Annual Dinner is scheduled for Friday, June 8, at 7:30 P.M. in the Rutland Room, preceded by cocktails at 6:30 P.M. in the adjoining Benjamin West Room.

Those wishing to present papers, which will be limited to ten minutes, should send four copies of the title and abstract to the Vice-President, Dr. Jane Russell, School of Medicine, Emory University, Atlanta, Georgia, not later than *March 1, 1951. It is imperative that the abstracts be informative and complete with results and conclusions in order that they may be of reference value and suitable for printing in the program and journals of the Association.* The following regulations for the preparation of abstracts and titles must be carefully followed to insure consideration of the paper for the program:

1. Abstracts may not exceed two hundred words, or equivalent space, exclusive of title. No footnotes or acknowledgments to sponsors can be published. References, if used, must be placed in the body of the

text. The abstract should consist of a single paragraph, if possible. Structural chemical formulas cannot be used.

2. The title heading must be arranged as follows:
   Line 1. Title, not to exceed fifteen words.
   Line 2. Author/s. The name of each non-member author collaborating with member-authors is to be followed by the phrase "(by invitation)." Names of non-members who are introduced, *i.e.*, who are not collaborators with member-authors, are to be followed by the phrase "(introduced by . . .)." The principal degree, *e.g.*, M.D., of each author should be given after his name.
   Line 3. Institution of origin and city in which institution is located.
3. The body of the abstract, typed double-space, should follow the heading. The original copy should be on bond paper. There should be three copies.
4. Abstracts should be letter-perfect, since there will be no opportunity for proof reading by authors.

(For details about the type of program, *see* the President's Letter to the Editor on page 1355 of the October 1950 issue.)

The Association for the Study of Internal Secretions

THE 1951 AWARDS AND FELLOWSHIPS

Fellowships

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship will not be awarded in 1951, so that it may accumulate toward a $5,000.00 Fellowship to be awarded in 1952.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

The Schering Fellowship in Endocrinology was established in 1949 and the first recipient was Dr. D. Laurence Wilson, who also received the 1950 Fellowship.

Association Fellowships are designed to assist men or women of exceptional promise in their progress toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $2,500.00, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study,

indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

Awards

THE E. R. SQUIBB AND SONS AWARD

The E. R. Squibb & Sons Award of $1,000.00 was established in 1929, and was given first in 1940 to Dr. George W. Corner; in 1941 to Dr. Philip E. Smith; in 1942 to Dr. Fred C. Koch; in 1944 to Dr E. A. Doisy; in 1945 to Dr. E. C. Kendall; in 1946 to Dr. Carl G. Hartman; in 1947 to Drs. Carl F. and Gerty T. Cori; in 1948 to Dr. Fuller Albright; in 1949 to Dr. Herbert M. Evans; and in 1950 to Dr. C. N. H. Long. In 1943 no award was given. A special committee of five members of the Association selects the recipient from among investigators in the United States or Canada, on the basis of outstanding contributions to endocrinology.

THE CIBA AWARD

The Ciba Award to recognize the meritorious accomplishment of an investigator not more than thirty-five years of age in the field of clinical or preclinical endocrinology was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; and in 1950 to Dr. Oscar M. Hechter. The Award is for $1,200.00. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $1,800.00.

NOMINATIONS

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the *Secretary, Dr. Henry H. Turner*, 1200 North Walker Street, Oklahoma City 3, Oklahoma, and returned to him *not later than March 1, 1951*.

The American Goiter Association

THE 1951 ANNUAL MEETING

THE 1951 annual meeting of the American Goiter Association will be held in Columbus, Ohio, on May 24, 25, and 26. The Deshler-Wallick Hotel, Broad and High Streets, will be the headquarters and every effort will be made by the hotel officials to make the meeting a success and the visit a very pleasant one. It would be well to see that reservations are made now.

The president, Dr. T. C. Davison, and the chairman of the Local Arrangements Committee, Dr. George Curtis, are planning an excellent meeting. It is hoped that there will be a large attendance of members.

GEORGE C. SHIVERS, M.D.,
Corresponding Secretary,
Colorado Springs, Colo.

The American Goiter Association

VAN METER PRIZE AWARD FOR 1951

THE American Goiter Association again offers the Van Meter Prize Award of three hundred dollars, and two Honorable Mentions for the best essays submitted concerning original work on problems related to the thyroid gland. The Award will be made at the annual meeting of the Association which will be held in Columbus, Ohio, May 24, 25, 26, 1951, providing essays of sufficient merit are presented in competition.

The competing essays may cover either clinical or research investigations; should not exceed three thousand words in length; must be presented in English; and a typewritten double spaced copy in duplicate sent to the Corresponding Secretary, Dr. George C. Shivers, 100 East Saint Vrain Street, Colorado Springs, Colorado, *not later than March 1, 1951.* The committee who will review the manuscripts is composed of men well qualified to judge the merits of the competing essays.

A place will be reserved on the program of the annual meeting for presentation of the Prize Award Essay by the author, if it is possible for him to attend. The essay will be published in the annual Transactions of the Association.

* * * * *